Nuclear mechanotransduction in stem cells

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Abstract

In development and in homeostatic maintenance of tissues, stem cells and progenitor cells are constantly subjected to forces. These forces can lead to significant changes in gene expression and function of stem cells, mediating self-renewal, lineage specification, and even loss of function. One of the ways that has been proposed to mediate these functional changes in stem cells is nuclear mechanotransduction – the process by which forces are converted to signals in the nucleus. The purpose of this review is to discuss the means by which mechanical signals are transduced into the nucleus, through the LINC complex and other nuclear envelope transmembrane (NET) proteins, which connect the cytoskeleton to the nucleus. We discuss how LINC/NET confers tissue-specific mechanosensitivity to cells, and further elucidate how LINC/NET acts as a control center for nuclear mechanical signals, regulating both gene expression and chromatin organization. Throughout, we primarily focus on stem cell - specific examples, notwithstanding that this is a nascent field. We conclude by highlighting open questions and pointing the way to enhanced research efforts to understand the role nuclear mechanotransduction plays in cell fate choice.

Introduction

There have been considerable recent advances in understanding how biochemical signals, as well as transcriptional and epigenetic control, affect development and stem cell fate [1–3]. In addition to these factors, the stem cell niche is a highly dynamic mechanical environment, and there is considerable influence of mechanical signals on stem cell function [4]. Indeed, during development and lineage specification, stem cells encounter and respond to extrinsic physical forces [5], yet there is currently limited understanding of how those forces regulate gene expression. Even less is known about how these processes can be exploited for better control of stem cell function in regenerative medicine.

Mechanical signal transduction begins at the plasma membrane, where forces can be transmitted through transmembrane receptor complexes like integrins, or through mechanosensitive ion channels such as Piezo1/2 [6], to initiate downstream signaling. Cell membrane mechanosensors also mediate contractility in the actin cytoskeleton, either through direct connection or by facilitating a change in molecules that affect contractility such as calcium. The polymerization state of actin, also influenced by contractility, controls serum response factor (SRF) signaling, which at least in some cells plays a role in cell signaling [7,8]. Moreover, the cytoskeleton also propagates mechanical stresses into the nucleus, affecting its molecular composition and physical arrangement [9,10], the structural organization and apico-basal polarity of the nuclear lamina [11], and molecular traffic across the nuclear envelope [12,13]. This relay of mechanical stress, called nuclear mechanotransduction [14,15], could occur in several ways, and involves several categories of macromolecular complexes: cytoskeletal-nucleus connections; the nuclear envelope; and chromatin. A greater understanding of the interplay between these complexes, and their potential effect on signaling and stem cell function, is the focus of this piece.

Nuclear envelope proteins: tissue-specific mechanosensitivity

Specialized protein structures responsible for the direct mechanical interfacing of the nuclear envelope and cytoskeletal components have now been identified and are collectively known as the linker of nucleoskeleton and cytoskeleton (LINC) complex

[16,17]. The LINC complex consists of SUN domain proteins that reside in the inner nuclear membrane, and Nesprins that reside in the outer nuclear membrane and span into the cytoplasm where they bind to various components of the cytoskeleton. The LINC complex effectively forms a bridge that allows for the direct transmission of mechanical signals from the cell surface to the nuclear envelope, on to the nucleoplasm and chromatin (**Figure 1**) [17]. A study that utilized a nesprin-2G FRET based tension biosensor demonstrated direct evidence of the LINC complex bearing mechanical load [18]. Additional evidence of the mechanical connection conferred by the LINC complex was presented in a study in which shear stress was applied to the cell surface, which subsequently stretched chromatin regions [19]. Here, it was demonstrated that a transgene inserted in a stretched region was transcriptionally upregulated due to stretch-enabled binding of RNA polymerase II. Both the chromatin displacement and transgene expression were abolished when SUN proteins were depleted, strongly suggesting the requirement of the LINC complex for force-induced transcription. We speculate that mechanical stress on the nucleus through LINC and other nuclear envelope transmembrane (NET) proteins, through its influence on transcription, is a primary mechanism governing how mechanical signals regulate stem cell fate choice (**Figures 1-2**).

How mechanics regulates fate choice almost certainly differs from tissue to tissue, as the enrichment of some components of cytoskeleton and nucleoskeleton vary with mechanical stresses. In particular, incorporation of Lamin A/C in the nuclear lamina has been shown to scale with tissue stiffness. Mechanically active or stress-bearing tissues such as muscle or bone have nuclei with high lamin A/C:B stoichiometry, while softer tissue that experience reduced external mechanical loads have low lamin A/C expression [20,21]. Lamin A/C also protects against DNA damage [22], which may play a role in aging and stem cell senescence. Lamin A/C assembly dynamically responds to force application via phosphorylation. Reduced force transmission to the nucleus increases Lamin A/C phosphorylation which subsequently solubilizes the protein [20]. This illustrates the force regulation of the nuclear lamins, and how that could potentially play a role in gene regulation and fate specification [23]. Importantly, however, a direct link between nuclear lamins and cell fate choice has yet to be firmly established.

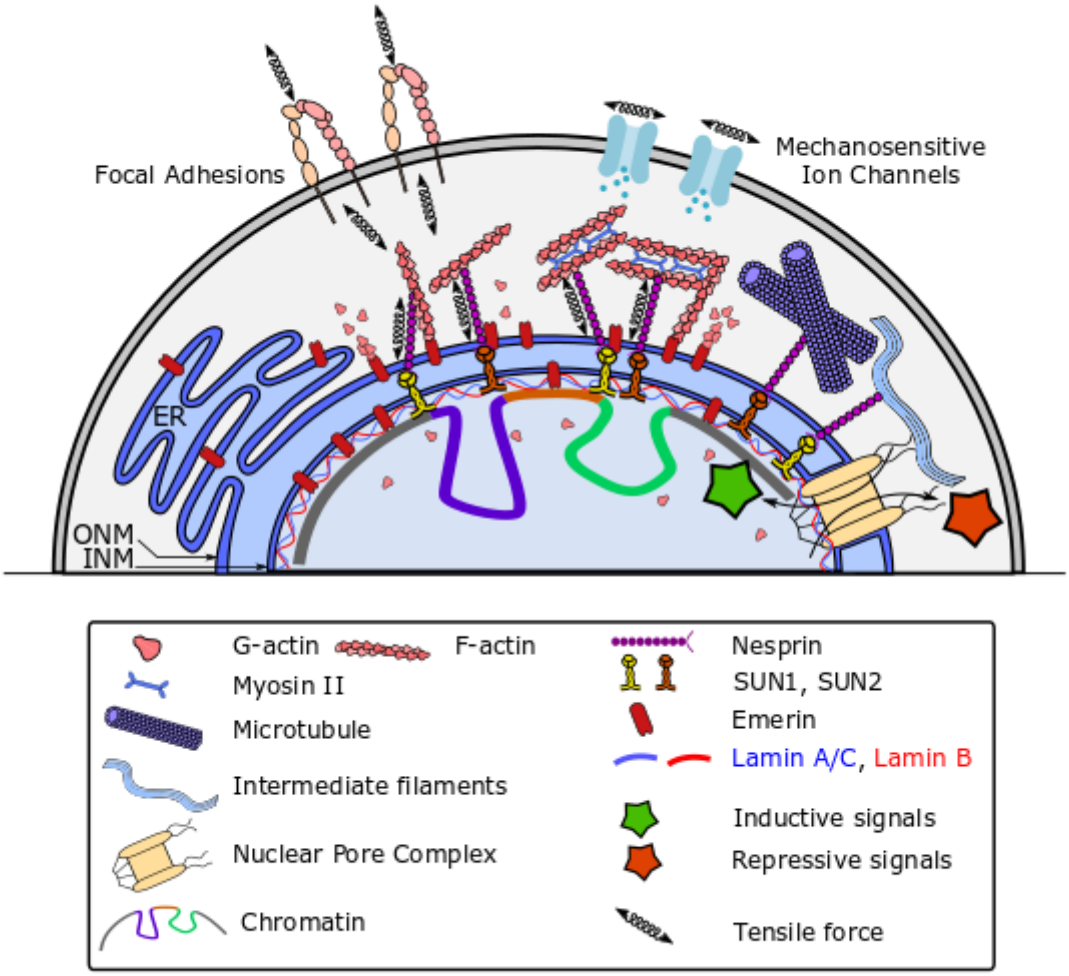

Figure 1. Factors in nuclear mechanotransduction

Forces applied to the cell, *e.g.* tensional forces sensed at the cell surface, are transmitted to the nucleus through a relay of mechanical stresses. In the cytoplasm, the cytoskeleton propagates stresses to the LINC complex. The Nesprins and SUN domain proteins that form the LINC complex connect to the cytoskeleton and can further transmit forces inside the nucleus to the nuclear envelope transmembrane proteins (NETs), the lamins, and ultimately, to chromatin. The LINC/NET proteins can also influence the nucleo-cytoplasmic trafficking of signaling factors through nuclear pore complexes. ER: Endoplamic Reticulum; ONM: Outer Nuclear Membrane; INM: Inner Nuclear Membrane.

LINC/NETs also have a tissue-specific composition [24]. The SUN1:SUN2 stoichiometry appears to vary across cell types [25], leading to different positioning in the nucleus based on whether they favor microtubule-based (SUN1) or actin-based (SUN2) movements [26]. A specific composition of LINC complex appears to define the range of mechanical strains to which a cell will respond, conferring a possible tissue-specific nuclear mechanosensitivity. It was shown, for example, that SUN proteins are required for the transduction of low magnitude strains in mesenchymal stem cells (MSCs) [27]. Engagement of the LINC complex then activated Focal Adhesion Kinase (FAK) and downstream ß-catenin signaling, which ultimately reduced adipogenic differentiation of MSCs [28]. Conversely, high magnitude strain was not transduced through the LINC complex but was instead sensed and transduced directly at focal adhesions, also activating FAK and ß-catenin activity [27]. Therefore, the expression of SUN domain proteins can determine the nuclear sensitivity to low and high magnitude strains, which in turn could activate different pathways.

Additionally, mechanical stress can play a key role in recruiting and stabilizing certain LINC/NET proteins while destabilizing others. For example, Emerin, which is an actin capping, transmembrane protein localized mostly in the inner nuclear membrane [16,29], exhibits a force-dependent phosphorylation in HeLa cells [30]. When force was applied to Nesprin-1, the nuclear envelope demonstrated a stiffening response, and it was shown that this was a result of Src-kinase-mediated Emerin phosphorylation driving the assembly of Lamin A. Other studies have suggested the possibility that Emerin phosphorylation recruits other NETs to assemble the LINC complex in response to force [17], and that Emerin itself is recruited at the outer nuclear membrane in response to cyclic strain in an acto-myosin dependent manner [31].

In cases of extreme mechanical stimulation, a stress response can lead to the disassembly of the LINC complex. For example, in human mesenchymal stem cells (hMSCs) under high frequency cyclic tensile strain, a rapid phosphorylation and turnover of SUN2 was observed within a few minutes leading to a decoupling of the nucleus from the cell [32]. In

this case, it is notable that the ratio of SUN1:SUN2 increases. Significantly, in mutant cells either lacking or overexpressing SUN2, the cell and nucleus remained weakly coupled, leading to higher strain-induced DNA damage. Optimal levels of SUN2 proteins, therefore, appear to be required to orchestrate an appropriate response to mechanical strain, whether that is concerning signal transduction or a weakening of the coupling between cell and nucleus to ensure DNA protection. In a similar manner, it was shown that high levels of Lamin A increase heterochromatin softening under high amplitude cyclic stretch, preventing DNA damage [33]. Cells with low levels of Lamin A in contrast show no softening but high levels of DNA damage. Ultimately, an appropriate balance of nuclear envelope proteins ensures cell-specific sensitivity to mechanical signals, and optimal downstream function, including regulation of gene expression.

LINC integrates mechanical signaling pathways to regulate gene expression

Beyond direct transmission of mechanical forces to the nucleus, the LINC complex also modulates signal transduction via interaction with transcription factors and other signaling factors. Both Nesprins and SUN proteins have been shown to interact with the important differentiation signal β-catenin, promoting its nuclear import [34,35]. On the nuclear side, Emerin also binds β-catenin, promoting its export and accumulation outside of the nuclear envelope [36]. The expression and activity of LINC/NET proteins, and perhaps most importantly their mechanical stability, therefore participate in regulating β-catenin signaling.

The nuclear envelope is also a site of actin polymerization under mechanical stimulation. For instance, assembly of an actin ring around the nucleus was observed in epidermal stem cells under cyclic stretch [31], and even under low magnitude strain in MSCs [27]. F-actin structures in the perinuclear area are relevant for several reasons: (i) they modify the balance of monomeric and filamentous actin, leading to a depletion of the pool of monomeric actin in the nucleus. This depletion affects RNA polymerase activity, chromatin remodeling complexes and long range chromosome territory movements [37,38]; (ii) they

can lead to localization of Emerin either in the cytoplasm or at the outer nuclear membrane, thus depleting it from the inner nuclear membrane [31,39]. The nuclear envelope-mediated balance of actin stress is a key regulator of SRF/Mkl1 (also called MRTF-A) signaling, which is central to a number of biological processes [40]. Mkl1 is bound to G-actin in the cytoplasm, shuttles to the nucleus upon dissociation from actin and acts as an SRF co-factor upon binding to DNA targets. Its nuclear export is G-actin dependent [7,40]. Emerin at the inner nuclear membrane, through its capability of binding nuclear actin, is necessary for Mkl1 nuclear accumulation. In many laminopathies where Emerin is either mislocalized or missing, Mkl1 nuclear translocation is impaired leading to, for example, cardiac developmental defects [41].

SUN proteins also seemingly regulate Mkl1 activation through signaling to RhoA. SUN1 and SUN2 appear to have opposite effects on RhoA activity, with SUN1 antagonizing RhoA activity and SUN2 stabilizing it, possibly through force-dependent regulation of Rho-GEFs [42]. The activity of ERK1/2 is also impacted by NETs, lamina proteins and their interactions. ERK1/2 can bind Lamin A [43], but this interaction itself depends on Emerin-Lamin A binding. As evidence, there have been numerous examples of abnormal ERK activity in Emery-Dreifuss muscular dystrophy and dilated cardiomyopathy, two diseases driven by mutations in *LMNA* [44,45]. The onset of these diseases and subsequent heart failure was prevented in mouse disease models by pharmacological inhibition of ERK [46,47].

Thus, the assembly of a specific LINC complex, and the stoichiometry, localization, and the mechanical stability of its proteins can significantly modulate pathways regulating gene expression. LINC proteins can directly bind signaling factors such as β-catenin, act upstream of signaling pathways such as in the case of RhoA/SRF/Mkl1, and modulate Lamin A - ERK interactions, which could have a strong impact on stem cell fate choice (see **Figure 2**). More generally, stem cell fate changes are known to be accompanied by changes in nuclear mechanics driven by changes in chromatin condensation states [29,48,49]. Taken together, the work we have discussed strongly suggests there may be an important relationship, via LINC/NETs, between nuclear mechanics and genome organization.

Mechanosensitive Nuclear Envelope Proteins mediate chromatin organization

For a given cell type, the spatial organization of the genome displays a number of invariant features, notably usually a strong correlation between gene activity and radial gene positioning [50,51]. This correlation can be generalized to a conserved compartmentalization of the genome between active and repressed regions [52], and a probabilistic (neither random, nor fixed) radial positioning of individual loci within these compartments [51]. Regions of the genome that are gene-poor, transcriptionally repressed and generally heterochromatic, are typically either found at the nuclear periphery, or wrapped around the nucleolus; regions that are gene-rich, actively transcribed, and generally euchromatic usually occupy a central ring, distant from the nuclear periphery. A better visualization of these compartments was recently achieved by a study mapping 3D genome organization in individual cells, using single-cell Hi-C technology [53]. Nevertheless, a notable exception to the radial positioning of heterochromatic and euchromatic regions is found in the rod cells of nocturnal mammals. These have an *inverted* nucleus, with heterochromatin in the center and euchromatin at the periphery, due to an absence of Lamin A and Lamin B receptor [54,55]. However, in most stem cells, positioning and genome compartmentalization of heterochromatin can be relatively well predicted by its proximity with the nuclear periphery [52]. The nuclear periphery and its interactions with chromatin would thus seem to be of crucial importance in the regulation of genome conformation and activity.

The chromatin regions closest to the periphery can be tethered to the nuclear lamina, forming interfaces known as Lamina Associated Domains (LADs), which were initially defined by DNA adenine methyltransferase (DamID) studies with Lamin B1 [52]. Through regulation of LADs and genomic organization, the nuclear envelope and its various components are likely to play a significant role in regulating gene expression as cells differentiate. Indeed, LADs are dynamically regulated during cell differentiation. A mapping of LADs in different cell states showed that as pluripotent cells differentiated into neural lineages, interactions of lineage genes with the lamina were reduced, allowing their

subsequent activation [56]. Also, during adipogenesis, the localization of *PPARG* away from the periphery correlated with increased transcriptional activity [57]. Lineage gene tethering and unlocking can be disrupted by abnormal lamina or NET protein expression. For instance, overexpressing muscle-specific NETs involved in tethering of lineage genes disrupted myogenesis by perturbing the sensitive spatiotemporal order of lineage gene expression [58]. It was also recently shown that the histone deacetylase complex HDAC3 acts as a chromatin tether [59]. This role of HDAC3 was necessary for the coordination of lineage gene expression in cardiomyocyte specification as HDAC3-null pluripotent cells precociously differentiated, attributed to reduced lamina tethering of lineage genes. These studies support the idea that chromatin-nuclear envelope interactions constitute an added regulatory layer, important in controlling the kinetics of gene expression during stem cell differentiation.

An open question is whether NET mechanosensitivity, and downstream effects on chromatin tethering and genome organization, can influence stem cell differentiation. If this is the case, Emerin likely plays an important role, as it is an actin capping NET that has many binding partners, including lamins, LINC and HDAC3 [16,60]. It is also involved in chromatin tethering and LAD anchoring at the nuclear periphery [61]. Several studies also implicate Emerin mechanosensitivity in laminopathies [39,62]. Moreover, recent evidence indicates that Emerin is vital to radial chromosome positioning, with chromosome territories mislocalized in DLD-1 colorectal adenocarcinoma cells cultured on soft substrates [63]. In this study, Src-mediated Emerin phosphorylation (on a different residue than those identified in [30]) led to the movement of some chromosomes territories to the nuclear interior, and to gene deregulation. Src inhibition selectively rescued some, but not all, chromosomes to their peripheral positioning. This selectivity is notable as it suggests chromatin region specific responsiveness to NET activity. In another study, it was found that co-depletion of Lamin A and Emerin also resulted in chromosome territory mislocalization and gene mis-regulation [64]. These studies further suggest that Emerin and the nuclear lamina are central for regulating chromatin spatial organization and gene expression.

In stem cells, few studies have so far bridged the gap between NET mechanosensitivity and genome organization. One important exception is a landmark study that demonstrated that in epidermal progenitor cells under strain, Emerin re-localized from the inner to the outer nuclear membrane. This resulted in the dissociation of lineage genes tethered to the nuclear lamina. Stretch-induced depletion of nuclear G-actin led to a drop in transcription followed by the Polycomb repressive complex targeting the untethered lineage genes [31]. Importantly, blocking Emerin re-localization *in vivo* using a conditional myosin heavy chain knockout led to precocious differentiation of epidermal progenitor cells, underlining the importance of mechanotransduction in controlling the timing of differentiation. This study and others [65] suggest that the dynamic mechanical properties of a stem cell niche can lead, through alterations in mechanical signaling, to significant alterations of cell state regulation.

Outlook for stem cells

LINC/NET proteins sense and transduce mechanical stress into the nucleus. By directly transmitting through the lamina to the tethered chromatin, and by interacting with signaling molecules, LINC/NET proteins such as Emerin are capable of impacting chromatin conformation, gene mobility, histone modifications, transcription factors, and ultimately the regulation of gene expression. Moreover, recent evidence has suggested that tissues possess a specific composition, stoichiometry and activity of LINC/NET proteins depending on the mechanical properties of that tissue. This unique fingerprint helps determine the sensitivity and response of each cell type to mechanical stress.

Putatively, each tissue could therefore use this tuning mechanism to alter its mechanosensitivity to the range of stresses it experiences, both during development and in homeostatic conditions. An unbalanced LINC complex (either by altered composition or by decoupling, **Figure 2**) could lead to a change or impairment in the cell-nucleus connection and a tuning or mis-regulation of signaling activity. This could have a profound impact on stem cells and development. Specific hallmarks of fate changes in embryogenesis such as

epithelialization and epithelial-to-mesenchymal transitions, and accompanying changes in cell and nuclear mechanics, subject the nucleus to significant mechanical stress. The change in mechanical stress is concomitant with high activity of many signaling pathways driving differentiation. Ultimately, the mis-regulation of LINC/NET arrangement could desensitize or oversensitize a stem cell to mechanical stimuli, and result in delayed or premature differentiation capacity by biasing the balance between self-renewal and differentiation, or potentially lead to senescence [66]. However, how LINC/NET affects stem cell function remains to be studied systematically. Also, as opposed to lamina proteins [20,67] the evolution of LINC/NET proteins expression throughout development remains to be mapped tissue by tissue.

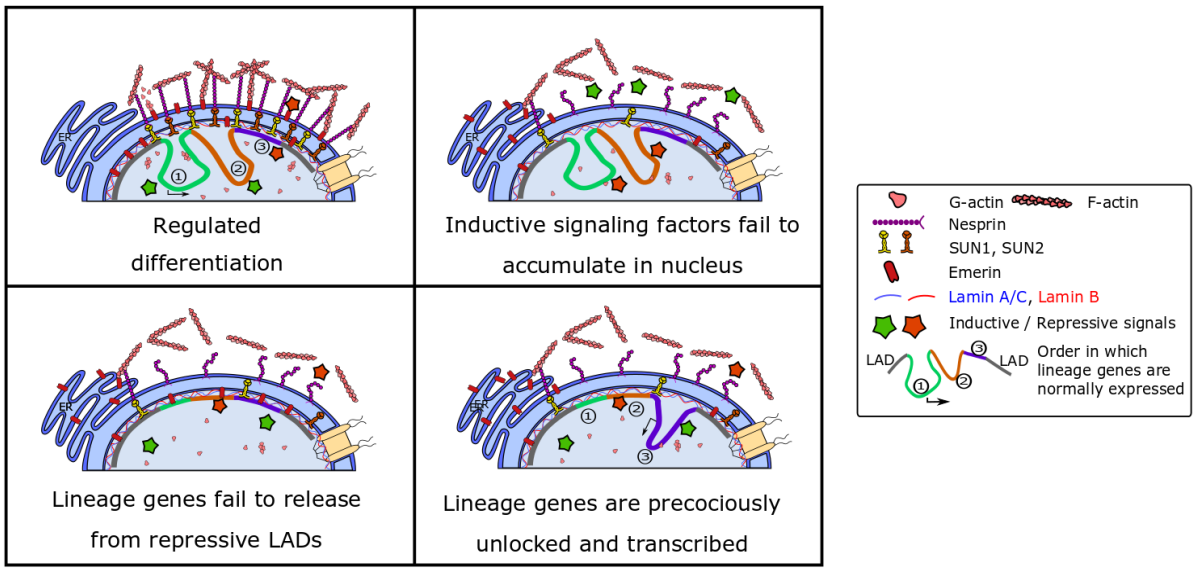

Figure 2. A balanced LINC complex is required for proper stem cell differentiation.

A balanced LINC comprises the following: (i) tissue-specific composition and stoichiometry of LINC/NET proteins (Nesprins, SUNs and Emerins represented here), (ii) normal activity of NETs as controlled by protein localization and post-translational modifications (not shown here), and (iii) mechanical engagement of factors allowing force transmission to the lamina and chromatin (for a given range of force amplitudes and frequencies). If one of these factors is missing, stem cell differentiation could be disrupted by the means illustrated above. Impaired differentiation could be a combination of these modes. First (top right), signaling factors (✡) such as β-catenin and MKL1, are not accumulated in the nucleus, leading to an imbalance in inductive and repressive factors. Second (bottom left), deficiencies in the NETs can delay/impair gene unlocking (through mis-regulation of histone modifications for instance). Lineage genes would therefore remain in Lamina-Associated-Domains (LADs), which tend to be repressive. While this does not necessarily lead to

absence of transcription, it can delay or limit the rate of transcription of locked genes. Finally (bottom right), a misregulation of tissue-specific NETs could also lead to premature unlocking of genes, resulting in early transcription of lineage genes and loss of spatiotemporal control in gene expression (bottom right). ER: Endoplasmic Reticulum.

As the relevant mechanical forces in stem cells are being better described [68,69], it will be interesting to specifically interrogate the composition and activity of LINC/NET proteins, and their role in mechanotransduction and cell fate choice. Mechanical stimulation assays along with specific perturbations to LINC/NET will be necessary to determine the role of mechano-coupling in stem cells during differentiation [70]. It is likely that the relationship between mechanical coupling and signaling will be lineage specific. Ultimately, it will be important to translate any knowledge gained from mechanical studies of stem cell differentiation *in vivo*, particularly how LINC/NETs and the nuclear lamins regulate stem and progenitor cell function in the development and maintenance of different organs.

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Annotations of selected references

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•• In this study, authors show that protein transport across the nuclear pore complex is a force-dependent process. They first build constructs of Mkl1 protein concatenated with domains of various mechanical stability, and tested their translocation rates as well as the force needed to unfold the protein domains. They reveal that nuclear import – but not nuclear export - correlates inversely with protein mechanical stability. This study constitutes the first in-depth exploration of mechanosensitivity of the nuclear translocation process.

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•• In this study, authors characterize the response of human mesenchymal stem cells to mechanical strain of increasing frequency by quantifying transcription, protein abundance, protein folding and post-translational modifications. They uncover the role of SUN2 turnover in decoupling the nucleus from the cell under high mechanical stress, which protects the cell against DNA-damage. They further demonstrate that an optimal level of SUN2 expression is necessary for this process, which is abrogated by overexpression or depletion of SUN2, highlighting the importance of LINC adaptation to a given mechanical environment.

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• In this study, authors investigate the mechanism behind the β–catenin-dependent mechanical blockade of adipogenesis in mesenchymal stem cells. They show that mechanically activated β–catenin associates with the nucleoskeleton (insoluble fraction of the nucleus) containing LINC proteins, nucleoporins and nuclear lamina. This association is transient but necessary for nuclear import. Depletion of SUN proteins decreases β–catenin association with the nucleoskeletal and its nuclear import. This constitutes an important step in understanding the mechanical regulation of β–catenin nuclear translocation and of mesenchymal stem cell differentiation.

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• Authors tracked expression and localization of *PPARG* gene, coding for an essential differentiation transcription factor, throughout adipogenesis in mesenchymal stem cells. They found that expression starts with a single allele, and progressively becomes bi-allelic. Transcriptional expression correlated with radial nuclear localization of each allele, with expression increasing as the locus became more central. This study strengthens the current literature linking gene positioning and gene activation in stem cell differentiation.

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• Authors developed cell lines with mutant Emerin similar to the ones found in atrial cardiac defects. Proteomic study showed that the mutants had slightly reduced affinity to barrier-to-autointegration factor (BAF). In mutant cells, Emerin was less abundant, causing in turn a decrease in SUN2. The mutant Emerin cell lines also displayed a weakened response to cyclic stretch or to substrate stiffness, having a lack of actin stress fiber formation. This study highlights the precise interplay of NETs and the LINC complex for proper response to mechanical signals, as well as suggests downstream genomic sensitivity, as Emerin and BAF are known to influence genome organization.

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• In this study, authors investigate the radial nuclear positioning of a gene-poor and a gene-rich chromosomes on soft matrices. They observe a shift of positioning away from the periphery, more pronounced in the gene-poor chromosome, and show that this can be attributed to Src-dependent phosphorylation of Emerin on soft matrices. The different sensitivities of chromatin regions to substrate softness is intriguing and suggests that future studies on mechanical regulation of chromosome positioning should take into account the specific interactions of each locus with the nuclear envelope.