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MECHANICAL REGULATION OF NUCLEAR STRUCTURE AND FUNCTION

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Abstract

Mechanical loading induces both nuclear distortion and alterations in gene expression in a variety of cell types. Mechanotransduction is the process by which extracellular mechanical forces can activate a number of well-studied cytoplasmic signaling cascades. Inevitably such signals are transduced to the nucleus and induce transcription factor-mediated changes in gene expression. However, gene expression can be also regulated through alterations in nuclear architecture, providing direct control of genome function. One putative transduction mechanism for this phenomenon involves alterations in nuclear architecture that result from the mechanical perturbation of the cell. This perturbation is associated with direct mechanical strain or osmotic stress, which is transferred to the nucleus. This review describes the current state of knowledge relating the nuclear architecture and the transfer of mechanical forces to the nucleus mediated by the cytoskeleton, the nucleoskeleton, and the LINC (linker of the nucleoskeleton and cytoskeleton) complex. Moreover, remodeling of the nucleus induces alterations in nuclear stiffness, which may be associated with cell differentiation. These phenomena are discussed in relation to the potential influence of nuclear architecture-mediated mechanoregulation of transcription and cell fate.

Keywords

nucleus; mechanotransduction; chromatin organization; LINC complex; osmotic stress

1. GENERAL INTRODUCTION AND HISTORY

In biological systems, mechanotransduction is defined as the process by which mechanical stimuli are converted to intracellular signals that control cellular physiology in both health and disease. A classic example is shear stress acting on endothelial cells lining the wall of a blood vessel. The force from the blood flowing past an endothelial cell evokes changes in cell morphology; cells reorient their cytoskeletons in the direction of flow and initiate signaling to neighboring cells, resulting in changes to local vessel elasticity (1, 2). In effect, the external stimuli are sensed by the cells, and those signals are transduced across the membrane, where they alter intracellular signaling pathways and result in a cellular response. Similarly, many other cells rely on mechanotransduction for maintenance of their phenotype and metabolic activities. This process is particularly relevant to cells of the musculoskeletal, cardiovascular, and pulmonary systems, which are exposed to significant

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physical forces under normal conditions (3). Importantly, mechanical factors may be involved in numerous pathological conditions including diseases such as atherosclerosis, osteoarthritis, osteoporosis, and many others (reviewed in 4). Recent years have seen growing evidence of the critical role of mechanical and other physical signals in controlling the fate of stem or progenitor cells (5-7). The mechanisms involved in mechanotransduction are complex and may depend on several cellular pathways involving the cell membrane, ion channels, cytoskeleton, and cell nucleus (reviewed in 8). This review focuses on how the nucleus reacts to extracellular mechanical forces, with specific attention to how these forces can influence nuclear architecture, gene regulation and cell fate.

Conceptually, the idea that development and differentiation are shaped by extrinsic (mechanical) forces is ancient. Aristotle thought that embryonic development was in part directed by the locomotive component of the soul. This so-called Aristotelian soul differed from the more traditional or spiritual concept of a soul in that it is generated through the (physical) mixing of male and female semen (9), producing a teleological driving force that gradually gestates that individual organism to its final form (10). Teleological reasoning such as this persisted for millennia. However, over time, the notion of the Aristotelian soul as a driving force was either replaced by a divine, perfecting force (11) or obviated altogether by the notion that organisms were preformed and grew with no need for ontological development or differentiation (10). In the mid-1800s, Darwin published his theory describing evolution as a slow and gradual process of descent with modification, resulting in stochastic variability capable of conferring selectable (morphological) advantages to promote survival or increased reproductive fitness of an individual. He included many observations of embryos, as he saw them as “picture[s], more or less obscured, of the common parent-form of each great class of animals” (12, p. 450). Along with this, and more central to this work, he was also among the first to promote a movement away from teleological thinking, suggesting that evolution (and embryology) need not be a process seeking to perfect a morphology, per se (11). This point was later elaborated on by Wilhelm Roux and Julius Wolff (13), in Germany, toward the end of the century.

Wolff was an orthopedic surgeon who conducted research into how the structure of bones changed with alteration of function owing to either growth or pathology. His ideas followed from those of Roux; namely, he maintained that life has two periods, an embryonic one characterized by trophic organ growth and differentiation, and an adult one in which growth and remodeling occurs only when stimulated by healing or (cellular) turnover (13). These stimuli, which Roux collectively referred to as developmental mechanics (11), had an overall effect on tissues irrespective of life period, resulting in their remodeling. Wolff postulated that there was a causal relationship between the physical forces acting on bones and the observed changes to both gross morphology and internal architecture. In particular, he predicted that remodeling of bone trabeculae followed the mathematical trajectories of the forces acting on them. He also went a step further, suggesting that these same mechanical signals could provide one plausible mechanism for Darwin’s theory of natural selection (13). Since the publication of Wolff’s work, the accuracy of some of his mathematical predictions have been called into question (reviewed in 14). Another common criticism is that his theory cannot be considered a law because other bones and tissues do not exhibit the universality sufficient to explain the phenomena he described in cancellous bone. Despite these detractions, his observations were made well in advance of the discovery of radiography and modern tissue biology techniques. Wolff’s work is thus deservedly recognized for its pioneering contributions to the burgeoning fields of orthopedics and tissue mechanobiology.

Although an understanding of the complete sequence of biophysical and molecular events involved in mechanotransduction is still incomplete, numerous discoveries in the past

decade have added to the evidence for the role of the cell nucleus in this process. Molecular complexes in the nuclear envelope were found to mediate a connection between the cytoskeleton (the cell's proteinaceous structural support network) and the lamin network (a structural component of the nucleoskeleton), the latter of which is located on the interior of the nuclear membrane. The LINC (linker of the nucleoskeleton and cytoskeleton) complex is of particular interest because lamins and their associated proteins play a role in genomic silencing-opening a hotline of sorts for mechanical communication directly to the genome, potentially literally shaping gene expression. This review examines the current state of nuclear and chromatin biology with a particular focus on how the nucleus responds to mechanical stimuli in terms of both homeostasis and how those stimuli affect gene expression and matters as fundamental as cell fate.

Throughout the 1800s, several prominent scientists theorized about nuclear function, but it was Theodor Boveri's work near the end of the century that experimentally confirmed the nucleus, and later the chromosomes, as the instruments of heredity in the cell (reviewed in 15). Boveri, who was closely assisted by his wife Marcella, is credited with several major shifts in biological thinking related to cancer and developmental biology. They were the first to report that cancer was clonal in nature, arising from a cell whose chromatin had been damaged (16). More relevant to this review is Boveri's chromosome theory, which was supported by experimental evidence in parasitic nematodes and sea urchins. The theory, in addition to definitively identifying the nucleus (and more specifically the chromosomes) as the material of heredity, demonstrated that genetic contribution is equal between parents and that all, not just a subset, of the chromosomes are transmitted from germ cells to somatic cells during normal embryonic development (17). This finding was especially important as it overturned the prevailing wisdom that developmental cell fate was specified by an incomplete sharing of the chromosomes among germ cells and the various types of somatic cells. In other words, it was thought that muscle cells received only the "muscle chromosomes," whereas brain cells inherited only the chromosomes relevant for brain function. Boveri also concluded that normal embryonic development involved a cooperation between the nuclear material and the cytoplasm. Introduced in the early twentieth century, these concepts are highly studied today and pivotal to any stem cell-based regenerative therapy. The importance of cooperation between regionalized factors [usually ribonucleic acids (RNAs) and proteins] in the cytoplasm and lineage-specific gene expression (nuclear factors) in specifying cell fate varies widely among species. The molecular and genomic revolutions of the late twentieth century brought genetic factors and gene-regulatory networks into prominence, especially where mammalian development was concerned.

2. NUCLEAR STRUCTURE AND FUNCTION

2.1. General Structure and Subnuclear Bodies

The nucleus houses the nuclear genome and provides a functional environment for the information that will maintain and reproduce that cell, and potentially the whole organism. Eukaryotes, whose word origin is Greek for true kernel or nucleus, are defined by a nucleus that is bound by a membranous envelope. This multilayer, selectively permeable covering is composed of the outer nuclear membrane (ONM) and the inner nuclear membrane (INM) that surround the perinuclear space and of the nuclear lamina on the nucleoplasmic side (**Figure 1a,b**). Nuclear envelope transmembrane (NET) proteins traverse either one or both of the nuclear membranes and represent a functionally diverse group of nuclear factors. Among these are the nucleoporins, which make up the nuclear pore complexes that bridge the cytoplasmic and nuclear compartments and are embedded ubiquitously throughout the nuclear envelope. These enable bidirectional, passive movement for small molecules and facilitate transport of larger (>40-kDa) ones (reviewed in 18). The innermost compartment of the nucleus is occupied by the nucleoplasm, the (nuclear) genome, and several types of

subnuclear bodies. The nucleolus is the largest of these, and nuclei sometimes contain more than one. It is made up of a number of proteins and ribonucleic protein complexes that are involved in ribosomal RNA synthesis and processing. An additional level of the nucleolus's organization differentiates it from other nuclear bodies; it is surrounded by specific regions of the genome termed nucleolar-associated deoxyribonucleic acid (DNA), which comprise approximately 4% of the genome (19). The other subnuclear bodies are mostly ribonucleic protein complexes that are active in RNA synthesis and processing, such as promyelocytic leukemia bodies, splicing speckles, and Cajal bodies. As none of these subnuclear bodies are membrane bound, it is thought that their structure (and therefore placement) is transient and determined by the electrostatic intermolecular forces that bind the protein and nucleic acid components of these complexes together (20).

2.2. Chromatin

The DNA in eukaryotic nuclei is packaged as chromatin, a complex of DNA and proteins. An organism's genome contains the total collection of genes and regulatory information, coded as a linear sequence of nucleic acid moieties, needed to maintain and reproduce that organism. Contained within most human nuclei are two copies of the genome, one descended from each parent. Each copy, which has approximately 3.1 billion nucleotide base pairs (bp) of DNA, containing >22,200 protein-coding genes and >9,900 ribosomal RNA genes, which code for >142,700 different gene products (21). Although the information is encoded linearly, the overall genome is not. Some chromosomes are gene rich, and others are gene poor. Some genes exist in clusters and are functionally coregulated because of their clustering (e.g., *Hox* genes, globins, protamines), whereas others exist bordered by what are known as gene deserts. With each bp estimated at 330 pm in size (22), the length of each copy of the human genome would be approximately 1 m, if all chromosomes were stretched out and aligned end to end. The diameter of most human nuclei is in the micrometer (to tens-of-micrometers) range. Chromatin proteins serve to compact and provide order to what would be otherwise a chaotic system.

Chromatin is organized into nucleosomes, each consisting of approximately 145 bp of DNA supercoiled around a core complex of four dimeric histone proteins: H2A, H2B, H3, and H4. Approximately 20-80 bp of linker DNA exist between each adjacent nucleosome, and this region is bound by histone H1 (**Figure 1b**). Although the core's structure has been mapped to near-atomic resolution (23), there is great debate about how H1 interacts with the nucleosome and whether this interaction is essential for the maintenance of higher chromatin order (24, 25). In low-ionic-strength solutions, a chromatin fiber has been measured to have a thickness of approximately 10 nm that is thought to fold back on itself to form higher-order, compacted chromatin. The interphase nucleus is likely a mixture of several types of folding that could account for an approximate 30-nm-diameter structure, and the manner of folding of a particular region is highly dependent on the internucleosomal linker length and the presence of linker histone H1 (24).

Much of the difficulty in modeling the structure of chromatin in the interphase nucleus comes from the dynamic nature of chromatin and the fact that many of the existing static models rely on experiments conducted in solution, which may not reflect the exact physiological environment. Moreover, the effect of factors such as ion concentration and molecular crowding on nuclear structure is not fully understood (26). Even with this as an open question, investigators have identified hundreds of chromatin and chromatin-associated proteins that either bind to DNA directly or help stabilize chromatin in higher-order, more compacted forms. This hierarchical packing allows the genome to be contained within the available space. However, regions of chromatin must be unpacked before they can be

accessed for transcription, and it is now becoming clear that exogenous mechanical factors may influence this process.

Chromatin generally exists in one of two forms: lightly compacted euchromatin or more highly compacted heterochromatin. The former conformation is generally thought to be permissive to transcription, whereas the higher levels of compaction achieved in heterochromatin inhibit transcription. These two forms of chromatin represent two distinct, although dynamic, compartments within the interphase nucleus. Apart from clear differences in levels of compaction, each of these states has fundamental molecular signatures that distinguish one from the other; these signatures involve covalent modifications to DNA and several amino acid residues from the termini of histone proteins from the nucleosome core particle (27). Most organisms, including humans, can methylate cytosine residues in DNA directly in a modification associated with transcriptional silencing in heterochromatin, gene promoters, and repetitive DNA sequences (28). Lysine (K) residues in histone tails, for example, are subject to numerous posttranslational covalent modifications such as methylation, acetylation, phosphorylation, ubiquitination, and SUMOylation. Initially, a so-called histone code was theorized to account for the general trends exhibited by genomic regions bearing these marks (29). On one hand, hyperacetylation of K residues in histones H3 and H4 are commonly associated with actively transcribed chromatin, whereas methylation of H3K9 and H4K20 tend to be seen in pericentromeric heterochromatin, which is transcriptionally silent. On the other hand, modifications such as lysine acetylation could influence chromatin topography directly by steric inhibition between neighboring nucleosomes. Additionally, these modifications create new binding epitopes that are recognized by effector proteins, potentially resulting in subsequent activation or silencing. Thus, a modification such as methylated H3K4, which tends to localize to the start of genes, was generally thought to promote transcriptional activation by recruiting a chromatin remodeling complex containing a plant homology domain, a peptide motif that recognizes and binds to the trimethylated H3K4 residue (30). However, plant homology domains are not specific to activating complexes (31). Moreover, if methylated H3K4 marks accumulate at sites other than the starting position of genes (32), this accumulation can signal transcriptional silencing of that locus (33). Thus, the overall impact of any modification requires contextual information including genomic location, cell-cycle stage, the presence of specific histone variants, the expression status of transcriptional coactivators and transcription factors, the net effect of other repressive marks such as DNA methylation, and the expression status of effector proteins (reviewed in 34).

Apart from the canonical histones, a number of histone variant proteins are involved in cellular processes such as chromosome segregation during mitosis, DNA repair, chromatin remodeling, and transcriptional regulation. In many cases, these variants are assembled into the histone core at the time of DNA replication as is H2A.Z in gene promoters and transcription start sites, and thus serve as a biochemical signpost for important genomic features (35). Other histone variants, such as CenH3, are inserted into larger spans of DNA and are localized to highly repetitive centromeric sequences. This histone protein is an essential component of the kinetochore, part of the molecular machinery that segregates chromosomes to daughter cells at the end of cell division (36). The incorporation of an H3 variant, H3.3, into the nucleosome cores was recently reported to be essential to the establishment of heterochromatin in paternal pericentromeric regions of mouse embryos (33). In some cases, histone variants are specific to cell lineages, such as the testes variant H3t, which is important in the histone-to-protamine exchange that takes place in the sperm chromatin of many animals (37). Although the amino acid sequence of the variants may not differ significantly from the canonical protein (33, 35), those small differences often localize to key positions in the nucleosome core particle such that variant-bearing nucleosomes package DNA in a different manner, thereby changing the topology of the chromatin fiber.

In some cases, this change stabilizes the chromatin fiber and provides a basic building block for a higher-order structure (e.g., H3.3 and heterochromatin) (33, 35); in other cases, this change produces a less stable structure, like promoting more efficient exchange of the nucleosome structure to the protamine toroids found in sperm nuclei, for example (37).

2.3. Chromosomes and Chromosome Territories

Beyond the transcriptional states of chromatin in the nucleus, there are other degrees of order. Chromatin is organized into chromosomes (whose name derived from the Greek word for colored body, because of the way that chromosomes stained in early cell preparations). Chromosomes condense during metaphase in mitosis, making them visible as discrete bodies by light microscopy. In this form, certain regional domains within a chromosome become obvious—for example, the telomeres and the centromeres. Each of these regions tends to be gene poor and full of repetitive DNA sequences. Centromeric chromatin contains an assortment of centrosome-specific proteins including CenH3, which are essential for chromosome segregation during cell division. Telomeres protect the ends of chromosomes from the shortening that occurs with every round of cell division, ensuring that no critical genes are lost (38). During interphase, the chromosomes decondense (compared with those in metaphase), but telomeres and centromeres maintain a heterochromatic conformation. Overall, however, chromatin maintains a specific nonrandom order within the nucleus, referred to as a chromosome territory (CT) (**Figures 1a,c** and **2**). These CTs were initially described in animal nuclei in the late nineteenth century by Carl Rabl, but it was Boveri who later coined the term in his theory of chromosome individuality (39, 40). It was not until the 1980s that Thomas Cremer's group provided definitive proof for the nonrandom arrangement of chromosomes in the interphase nucleus, initially using ultraviolet microirradiation (41) and then in situ hybridization (42) techniques.

Each territory is composed of numerous megabase domains, the architectural units that constitute chromosomes in the mammalian interphase nucleus. Each of these megabase domains has a length of approximately 0.5-1 megabase pairs (Mbp) of DNA (40). Initially, this number was attributed to foci of replicating DNA visualized in the nucleus (43), but recent studies of genomic organization using high-resolution sequencing techniques corroborate this value (19, 44). The radial arrangement of specific CTs in the nucleus is also not random: Gene-dense chromosomes localize to the interior of the nucleus, whereas gene-poor chromosomes are located more peripherally (45-47). This arrangement has been evolutionarily conserved among vertebrates, but other corollaries, such as transcriptional activity and replication timing of certain subregions of chromosomes, have also been reported (reviewed in 40). In addition to radial arrangements in the nucleus, chromosome neighborhoods, which are specific arrangements between a subset of chromosomes, have been described with some tissue or cell-type specificity (48). The majority of these studies used fixed preparations that provided only snapshots in time and space, and, as such, they have not shed any light on how these arrangements are maintained or what mechanism is responsible for their positioning.

Another hotly debated matter with respect to CTs and nuclear architecture centers on the remaining space, or lack thereof, in the nucleus. One model posits that along with the chromatin in CTs that comprise a highly ordered interconnected network, there is an additional interchromosomal compartment (IC), which is largely DNA/chromatin free and contiguous with the nuclear pore complexes (49) (**Figure 1c**). This IC is the location where splicing speckles, transcription factories, and other subnuclear bodies are found. Transcription factories, as the name implies, are sites that feature several active clusters of RNA polymerase together with all the necessary cofactors that comprise the preassembled transcriptional machinery(50). This notion became quite popular with several reports of

gene-dense regions that form discrete chromatin loops outside the periphery of their respective CTs. The human major histocompatibility complex (51) and the mouse *HoxB* cluster (52) are two such examples. Upon activation-by interferon stimulation in the former case and by developmental timing of cluster expression in the latter case-a decondensation event was triggered at these loci, resulting in their constituent genes being pushed out into the IC to be transcribed. Investigators have also reported other looping phenomena, named chromosome kissing events, that involve the coregulation of disparate genomic loci, sometimes on different chromosomes (53). The competing model suggests a more dynamic picture, in which interactions between neighboring CTs are far more frequent than the rare looping events at a handful of loci. As such, any one snapshot in space and time shows a more intermingled state between adjacent CTs (54).

2.4. The Nuclear Lamina, the LINC Complex, and Their Associations to the Genome

Just interior to the INM, the lamina is a structural layer of intermediate filaments composed largely of lamin protein products from three evolutionarily conserved genes, *LMNA/C*, *LMNB1*, and *LMNB2*, and the proteins that associate to them, the lamin-associated proteins and lamin receptors (**Figure 1a**). The mechanical and functional properties of the lamina can vary greatly among cell types depending on the relative ratios of lamin splice variants that constitute the intermediate filaments (55). Sometimes referred to as the nuclear scaffold, lamins and their associated proteins can interact with the chromatin (directly at the DNA level or through a chromatin or chromatin-associated protein) by directly binding nuclear scaffold or matrix attachment regions. These regions usually flank the coding sequences of genes and insulate them from the effects of neighboring genomic regulatory elements (56, 57).

Embedded within the nuclear envelope are a series of proteins that facilitate force transfer throughout the cell (58). The LINC complex describes several classes of molecules that are localized to the INM and ONM and provides a functional link between the support structures of the cytoplasmic and nucleoplasmic compartments (**Figure 1a,b** and Table 1). They were discovered through a series of genetic screens in *Caenorhabditis elegans* and *Drosophila*, in which mutants displayed nuclear displacement phenotypes (59). Klarsicht/ANC-1/Syne-1 homology domain proteins (KASH-domain proteins) form the link between the ONM and elements of the cytoskeleton. Nesprins 1 and 2 bind actin filaments as well as several of the motor proteins of the microtubule network (reviewed in 59). Nesprin 3 binds plectin, which connects it to networks of intermediate filaments (60). On the INM side, Sad1 and UNC84 domain proteins (SUN-domain proteins) Sun1, Sun2, and Sun3 bind the lamina in the nucleoplasm (reviewed in 59 and 61). Other Sun proteins, including Sun1 η , Sun3, and sperm associated antigen 4 (Spag4) are expressed only in the spermatogenic lineage, and have been reported to be important in the association between the genome and nucleoskeletal manchette microtubules (163, 164). In the perinuclear space, the Nesprins' KASH domains interacts with the SUN domains, establishing the LINC and maintaining the perinuclear space. Emerin protein (EMD) is a transmembrane protein that localizes to the ONM, where it can form an association with the microtubules and the microtubule organizing center. When localized to the INM, EMD binds the lamina. To date, however, communication between INM- and ONM-bound EMD has not been reported (62). Mutations in lamins and other lamina components have been implicated in a class of pathologies termed laminopathies, such as Hutchinson-Gilford progeria syndrome (63) and Emery-Dreifuss muscular dystrophy (64). Cells in these disorders display a classic nuclear dysmorphology, altered nuclear and cellular stiffness (65), and disorganization of heterochromatin domains in the nucleus (66). As the LINC complex plays an important role in nuclear placement and anchoring and as lamins are important not only for bulk chromatin

organization in the nucleus but also for transcriptional regulation, it is a key to how mechanical signals traverse compartments in the cell.

Domains of highly compacted chromatin have been described at the nuclear periphery and bordering the nucleolus. In higher eukaryotes, associations with the nuclear lamina have been implicated as part of a long-term silencing mechanism for large regions of the genome (44, 67). All nuclear lamins contain domains in their C termini that bind chromatin with a high efficiency through direct association with histone proteins (68) or through interactions with B-type lamin receptor and heterochromatin proteins HP1 α and HP1 γ (69). However, repression is thought to be cooperative between the C terminus and the rod domain of LNMA only, which targets the promoter regions of genes (70). Through development, as cells differentiate from a more pluripotent (stem cell- or progenitor cell-like) state to a more specified state, the transcriptional demands on the genome change. Genes critical for the maintenance of pluripotency become silenced, and lineage-specific markers become active. This phenomenon has been recently shown to be at least partially mediated through interactions with the nuclear lamina. In mouse embryos, cells that will form placenta were observed with large domains of higher-order chromatin forming adjacent to the nuclear lamina (71), whereas those that retain a pluripotent state-which is necessary to specify all the cell types making up the embryo-maintain their genomes in a state of lower compaction. A dynamic situation is set up whereby segments of the genome that require activation come away from the lamina, whereas the newly silenced loci can become tethered there (40, 72, 73). Three independent 2008 studies used the LacO-LacI system to direct specific loci to the nuclear lamina and reported varying results (67, 74, 75), suggesting that this may be a two-step process involving an initial silencing event followed by a repositioning to the lamina for long-term silencing.

Conventional wisdom has largely held that constrained diffusion is the only means of movement in the interphase nucleus (76, 77). Thus, as with the looping and chromosome kissing events, no definitive mechanisms have been delineated to explain this motion of chromatin with respect to the lamina; however, two groups report interesting findings that shed new light intranuclear repositioning of gene loci and CTs (78, 79). The Belmont group (78), using live-cell time-lapse imaging, observed an actin/myosin-dependent displacement of a green fluorescent protein-tagged transgenic locus from the nuclear periphery to the interior upon induction during interphase. Myosin is an adenosine triphosphate (ATP)-dependent motor protein that tracks along the actin cytoskeleton through the cytoplasmic compartment. Interestingly enough, depriving the cell of ATP leads to changes in chromatin organization in the nucleus, but this has been attributed largely to the ATP dependence of numerous chromatin and nucleosome remodeling complexes that are active in the nucleus (76, 77). The Bridger group (79) reported a nuclear /actin/myosin-dependent mechanism of chromosome and gene locus displacement, but their findings suggest a dependence on entry or exit from the cell cycle (80, 81), as well as an intact lamina (82), as cells with mutations in *LMNA* linked to Hutchinson-Gilford progeria syndrome fail to show the same chromosome dynamics.

In mitotic cells, changes in intranuclear chromosome position could be explained by a mechanism in which an affinity-based process promotes interactions between mitotic chromosomes and specific NET proteins (83). During mitosis, the nuclear envelope breaks down into smaller vesicles in early prophase, and chromosomes condense, allowing their free movement in the cell. During this time, certain vesicles enriched with chromatin-binding NET proteins have an opportunity to associate with various targets on the mitotic chromosomes, including specific epigenetic marks, and a multitude of chromatin-associated proteins. When the nuclear envelope reforms in the subsequent interphase, the chromosomes that were high-affinity targets for the chromatin-binding NET proteins are tethered to the

nuclear periphery, whereas others are forced into the interior of the nucleus by steric hindrance once chromosomes decondense. A recent proteomic analysis identified hundreds of novel NET proteins (84). A screen focused on identifying factors involved in genomic architecture involving just 10 of these yielded two NET proteins that demonstrated the capacity to reposition a gene locus within the nucleus. Given these early results, it is tempting to speculate whether a NET protein-based mechanism could be involved in the changes to 3D genomic architecture observed during differentiative cell divisions.

3. MECHANICAL/STRUCTURAL PROPERTIES OF CELLS

3.1. The Cytoskeleton

The proteins of the cytoskeleton exist in the cytoplasm as a population of dissolved monomers in dynamic equilibrium with large polymeric cytoskeletal structures. As a result of their dynamic nature, cytoskeletal structures are at once robust and easily reconfigured. Tubulin monomers assemble into thick hollow tubes named microtubules that resist bending and are therefore capable of bearing compressive loads. Microtubules radiate throughout the cell from a microtubule organizing center located next to the nucleus. They contribute to the mechanical integrity of the cell and also provide a network of tracks along which organelles are driven by motor proteins (85). Intermediate filament proteins form fibrous structures that are flexible but resistant to fracture and extension (86). Actin monomers remain in solution as long as they are bound to thymosin. However, another actin-binding protein named profilin can displace thymosin and make actin available for assembly. Therefore, the cell can initiate rapid assembly of polymeric actin structures by producing profilin (87). This rapid actin assembly allows the cell to apply forces to its environment. The nature of the assembled structure and the forces generated depend on other actin-associated proteins. Actin filaments associate with one another in parallel to create thick bundles when they are cross-linked by α -actinin (88). Myosin proteins can bind to these filaments and drive them past one another to exert tension through the filament bundle (89). In addition, Filamin cross-links actin filaments at an angle so that they assemble into a gel (90). Taken together, the cell can push on its environment by assembling an actin gel adjacent to the cell membrane, for example, during the extension of a lamellipodium. The mechanical properties of the substrate affect the organization of the actin cytoskeleton. Stiff substrates increase bundling and tension in actin fibers (91). Substrate stiffness influences differentiation in a manner that depends on the activity of the myosin motor proteins that create tension in actin fibers (92). Therefore, physical properties of the extracellular matrix (ECM) influence events occurring within the nucleus via the cytoskeleton.

3.2. Extracellular Matrix: Transfer of External Strain to Cells and Subcellular Compartments

Physical stresses in the cell environment propagate through a series of mechanical connections to the nuclear interior. Bulk compression of cartilage, for example, causes shape changes in the nuclei of chondrocytes in an actin-dependent manner (93). Extracellular mechanical stresses act on the cytoskeleton via integrins, cell-surface proteins that connect to both the cytoskeleton and the ECM. For example, the nucleoli within fibroblast nuclei were found to reorient along the direction of tension that was applied through beads bound to the cell surface via integrins (94). This behavior implies the presence of a mechanical connection spanning the nuclear envelope. As noted above, the LINC complex spans the perinuclear space and connects all three cytoskeletal components (60, 95, 96) with the nuclear lamina (97, 98). Transmission of force from the cytoplasm to the nuclear interior requires intact LINC complexes, although the activation of mechanotransduction processes originating at focal adhesions may not (58). In addition to transmitting cytoskeletal stresses to the nucleus, LINC complexes allow the nuclear lamina to contribute to the overall

stiffness of the cell (55, 99). Mechanical activation of nuclear factor κ B was compromised in *Lmna*-deficient mouse embryo fibroblasts, suggesting that the lamina plays an important role in the mechanotransduction of extracellular stresses into gene activation (99). The nuclear lamina not only connects the nucleus to cytoskeletal stresses but also protects it from them. *Lmna*-deficient cells deformed more during cell-stretching experiments than did their wild-type counterparts and had decreased viability under cyclical mechanical strain (99). EMD is another protein that resides at the interface between the nucleus and the cytoplasm. It binds lamin, actin, and components of the LINC complex as well as several transcriptional repressors. *Emd*-null cells have approximately normal mechanics but impaired mechanotransduction, suggesting that EMD plays a role in communicating mechanical stresses to the nuclear interior (100). LNMB1's contribution to the overall mechanical properties of the nucleus is complex. On one hand, its deletion does not impact the overall stiffness of the lamina and only leads to minor aberrations in lamina architecture, as evidenced by the increased incidence of localized blebbing, coinciding with focal reductions of LMNA and C in the nuclei of *Lmnb1*-deficient cells (101). On the other hand, cells lacking *Lmnb1* display a nuclear rotation phenotype where the nucleus and its contents rotate freely, confined by the cytoskeleton, in an ATP-dependent fashion. It is thought that the loss of LMNB1 disrupts the organization of Sun proteins or other INM proteins required for the localization of the Nesprin proteins to ONM (102).

3.3. Mechanical Properties of Tissues, Cells, and the Nucleus

Biological tissues exhibit complex mechanical properties, and a range of sophisticated mechanical theories have been developed to describe these properties. Of the major tissue types, only bone can reasonably be approximated as a linearly elastic material subject to small strains (103). Large strain effects must be explicitly accounted for in many scenarios, including traumatic brain injury, which occurs at intermediate levels (at strains of 20%) (104). Some tissues, such as the annulus fibrosus of the intervertebral disc or the arterial wall, are anisotropic because they are reinforced by aligned fibers (105, 106). Biological tissues often exhibit complex rate dependence characterized by a spectrum of time constants rather than by a single value (107). In cartilage, rate dependency arises from the flow of fluid through the porous solid matrix, a process that is quantitatively described by biphasic theory (108). The flow of fluid is, in turn, influenced by the presence of fixed electrostatic charges in the matrix, an effect that has been modeled using triphasic theory (109). Stress and strain distributions within biological tissues can be estimated using continuum biomechanics by careful application of the tools described above. The local stress-strain and fluid-flow states within a tissue determine the physical environment of cells at that point. Multiscale modeling can be used to describe this physical environment and how it is modulated by the pericellular domain (110-112).

Cells exhibit viscoelastic material properties determined by their cytoskeletal organization, which is, in turn, influenced by external stimuli. For example, osmotic loading stimulated calcium signaling in chondrocytes, which, in turn, triggered disassembly of the actin cortex and a reduction in cell stiffness (113). Conversely, endothelial cells exposed to shear stress increased actin organization and stiffness (114). Mechanotransduction may alter cell physiology by stimulating conventional biochemical signaling paths via ion channel activation (115) or activation of other membrane-bound receptors (116, 117). Alternatively, however, mechanical stresses are transferred from the ECM directly to the cell nucleus via the LINC complex (58, 118). The consequences of mechanical stresses in the nucleus depend in part on the mechanical properties of the nucleus.

Mechanically, the nucleus is composed of two major structures, the lamina and the nucleoplasm within it. In creep experiments, the nucleoplasm is softer and more viscous than the nuclear lamina. Both structures exhibit power-law rheology under creep loading;

i.e., no single time constant dominates the response (119). This type of rheological behavior is common in gels and other materials that exist close to the transition between solid and liquid states (120). Whereas lipid bilayers are commonly described as two-dimensional liquids, the nuclear lamina is a two-dimensional solid (121); the distinction is that the solid lamina can resist shear stress, as evidenced by the buckles that arose in the lamina when it was aspirated into a micropipette in the study by Dahl and colleagues (119). When tested independently of the rest of the nucleus, the nucleoplasm behaves as a Maxwell fluid. The corresponding Young's modulus was found to be 36 Pa in a study that employed a 100- μm -diameter bead loaded only by Brownian motion (122) and 250 Pa in a study that employed a 500- μm -diameter bead loaded via an externally applied magnetic field (123). This contrast suggests that the mechanical properties of the nucleoplasm may depend on the length scale on which they are measured. The structural stiffness of the nucleus derives primarily from the nuclear lamina. Experiments that loaded the whole nucleus via micropipette aspiration (124) or unconfined compression (125, 126) observed solid viscoelastic behavior, with moduli in the kilopascal range-i.e., at values much higher than those of the nucleoplasm. In summary, much of the behavior of the nucleus under physical stress can be explained by modeling the nucleus as a soft, viscoelastic gel encapsulated in a stiff viscoelastic membrane and connected to the ECM via cytoskeletal structures and integrins.

3.4. Mechanical Perturbation of the Nucleus: Deformation, Osmotic Effects, and Cell Morphology

Mechanical and osmotic stresses can influence the nucleus directly as well as through indirect effects on the cytoskeleton (127). For example, intervertebral disc cells subjected to uniaxial stretch in monolayer culture exhibited nuclear strain that was lower than the applied cytoplasmic strain; this observation was attributed to the fact that the nucleus is typically stiffer than the cytoplasm (128). Epithelial cells experience shear stress in situ because they are loaded by blood flow. Shear stress causes the nucleus to flatten, elongate in the direction of flow, and stiffen (125), probably owing to upregulation and redistribution of A-type lamins (129). The elongated shape persists after the nucleus is isolated from the cell, demonstrating that the nucleoplasm is not a solid capable of elastic rebound (125). When human mesenchymal stem cells were cultured on nanoscale gratings, their nuclei aligned with the direction of the grating because the topography of the grating stimulated rearrangement of the actin and intermediate filament networks in the cytoskeleton (130).

In addition to mechanical stress, osmotic stress is a potent mediator of mechanotransduction in connective tissues such as cartilage and intervertebral disc (131), where it is coupled to compressive stress by triphasic effects (109), and it affects the geometry and other physical properties of the nucleus in several important ways. Osmotic stress alters cell volume (132) and therefore alters the concentration of macromolecules inside the cell. The chromatin inside the nucleus dilates with changes in macromolecule concentration (133-135) as a consequence of the thermodynamics of macromolecular crowding (136, 137). Therefore, under hypo-osmotic stress, the nucleus expands until the nuclear lamina draws taut around the nucleoplasm and prevents further expansion. Under hyperosmotic stress, the nucleoplasm contracts, and the nucleus shrinks. In addition, hyperosmotic stress results in marked chromatin condensation (**Figure 3**). Since the nuclear lamina resists changes in area, it buckles as the nucleus shrinks, forcing the nucleus into a convoluted shape (134, 138) (**Figure 3**). The increased macromolecular crowding that accompanies hyperosmotic stress impedes diffusion of macromolecules in the cytoplasm. However, the effect on diffusion within the nucleoplasm is negligible because it is offset by reorganization of the chromatin within the nucleus (135, 138). Chromatin is interdigitated by channels and pores of the IC (139) that accommodate rapid motion of macromolecules (140), and these channels enlarge as the chromatin contracts (49, 135), offsetting the effects of increased macromolecular

crowding. The change in nuclear geometry in the absence of changes in intranuclear transport leads to increased nucleocytoplasmic transport of inert macromolecules under hyperosmotic stress (135). This is significant because transport of macromolecules from the cytoplasm to the nucleus is a key activation step in several intracellular signaling pathways. Therefore, osmotic stress may enhance signaling along these pathways through a combination of purely biophysical processes.

3.5. Differentiation and Migration

There is an intriguing relationship between cell fate and compliance of the nucleus. *Lmna* expression increases as cells differentiate (141), making the nucleus stiffer (**Figure 4**) (142). Inside the nucleus, chromatin is generally diffuse in embryonic stem cells but condenses into higher-order structures as the cells differentiate (143). This pattern is also evident in the early mouse embryo, as higher-order chromatin structure begins to accumulate at chromocenters and around the periphery of the nucleus in cells that will adopt a trophectodermal fate in contrast to cells on the interior of the embryo that maintain a pluripotent program (71). As differentiation proceeds, epigenetic modifications accumulate throughout the genome in a manner that silences genes associated with self-renewal and pluripotency in favor of terminally differentiated genetic programs (144). This phenomenon suggests that chromatin organization may govern gene expression and hence cell fate, although it is not certain that the association between chromatin condensation and silencing is causal (20). A deformable nucleus facilitates normal stem cell behavior because it accommodates motion of the cell through tortuous three-dimensional environments. The nucleus is the largest, stiffest organelle in the cell and hence is the primary impediment to motion as cells squeeze through tight spaces. Efficient migration requires condensation of chromatin (145), which also helps the nucleus fit through small spaces. Other cells that migrate through the ECM to fulfill their functions also have low levels of A-type lamin expression, which make their nuclei more deformable (101). For example, the human neutrophil has low levels of lamins, lamin-associated proteins, lamin receptors, and *EMD* expression that allow it to pass through small gaps between endothelial cells in blood vessel walls (146). Similarly, cancer cells invade tissues as they metastasize, and again, nuclear changes that promote deformability occur. Although cancer cells are a heterogeneous population, lamin expression is reduced in many types of cancer (147, 148). The impressions made by individual extracellular collagen fibers can be seen in the nuclei of cancer cells as they invade the ECM, suggesting that the fibers physically compress the nucleus as it passes between them (149). Nuclear deformation during cell migration provides a clear and compelling example of a physiological process that depends on the mechanics of the nucleus, but the interaction is passive. One of the most daunting and important challenges in the field of nuclear mechanics today is demonstrating that a mechanical stress can propagate from the ECM, through the cytoskeleton, across the nuclear envelope, and into the chromatin, where it physically deforms the DNA or associated proteins, thus changing gene expression.

3.6. Mechanical Regulation of Gene Expression

Physical stresses can act on the nucleus and nucleoplasm and also cause a change in gene activity, although a causal relationship between mechanical stimuli and a biological response cannot always be shown. For example, the gene *CTFR* moves from the transcriptionally repressive nuclear periphery into the center of the nucleus upon activation. However, artificially inducing this relocation does not activate the gene, indicating that these are molecularly separate events and that relocation is a consequence of activation rather than a cause (150). Microtubules induce dynamic fluctuations in the nuclear envelope as they push against it, and these fluctuations “agitate” the chromatin within, causing different chromatin domains to move relative to one another (151). Chromatin topology influences

gene regulation (139, 152, 153), so these microtubule-pushing forces may alter gene activity as they reorganize the nuclear interior, but direct evidence for this is lacking. Conformational changes occur in several cytoskeletal proteins when a cell is tensed, but none have been reported in chromatin (154). Subnuclear structures such as Cajal bodies and promyelocytic leukemia bodies exist within the IC, and the dynamics and architecture of the surrounding chromatin determine their motion (155). This implies that the RNA-processing interactions between these bodies and the surrounding chromatin are influenced by reorganization of that chromatin under mechanical stress. Mechanical strain rapidly activates tenascin-C gene expression in fibroblasts independent of protein synthesis or paracrine effects, making this a good candidate for investigation as a gene that is directly activated by mechanical stress (21).

4. SUMMARY AND CONCLUSIONS

There is significant evidence that physical signals can be transmitted readily from the ECM to the cell nucleus. In the case of mechanical stresses, there is a direct molecular link between the ECM molecules and the nucleoplasmic compartment. Integrins span the cell membrane and connect the ECM molecules to various cytoskeletal elements which in turn associate both directly and indirectly to the LINC complex, whose Sun protein components form the final link to the nucleoplasm and the nuclear lamina (e.g., 93, 94, 156, 157; Fig. 1). Osmotic stress, in contrast, can alter nuclear size, structure, and physical properties through alterations in the physicochemical characteristics of the cytoplasm, potentially circumventing a direct mechanical link (reviewed in 138). In either case, such changes in nuclear and chromatin structure can have a profound effect on biological events such as gene transcription, nucleocytoplasmic transport, and protein synthesis.

The degree of interconnectedness of the cell's support structures throughout cytoplasmic and nuclear compartments provides a means for direct communication and force transfer. That these forces lead to the agitation of chromatin domains provides tempting grounds for speculation as to how mechanical forces may result in CT movements in an interphase nucleus. Alternatively, the mechanical stimulus may simply provide a signal to the cell, causing it to either enter or exit the cell cycle, with commensurate changes in chromatin and nuclear organization similar to those described by Bridger and colleagues (80, 81). Understanding how NET proteins can specifically target gene loci and entire chromosomes to specific subnuclear regions (83) is also an important open question to be answered. As the tools for studying intracellular biomechanics improve, the connection between physical stresses, nuclear architecture, and gene activity is becoming more apparent, and our increased understanding of this connection opens up the possibility of a new realm of cell signaling pathways comprising biophysical as well as biochemical events. As these discoveries accumulate, they add to the importance of nuclear mechanics in our overall understanding of the cell.

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LIST OF ABBREVIATIONS

ATP	adenosine triphosphate
bp	(nucleotide) base pairs

CT	chromosome territory
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EMD	emerin protein
H1/H2A/H2B/H3/H4	histone proteins
IC	interchromosomal compartment
INM	inner nuclear membrane
K	amino acid lysine; K20, e.g., represents lysine, residue 20
LINC	linker of the nucleoskeleton and cytoskeleton
NET protein	nuclear envelope transmembrane protein
ONM	outer nuclear membrane
RNA	ribonucleic acid

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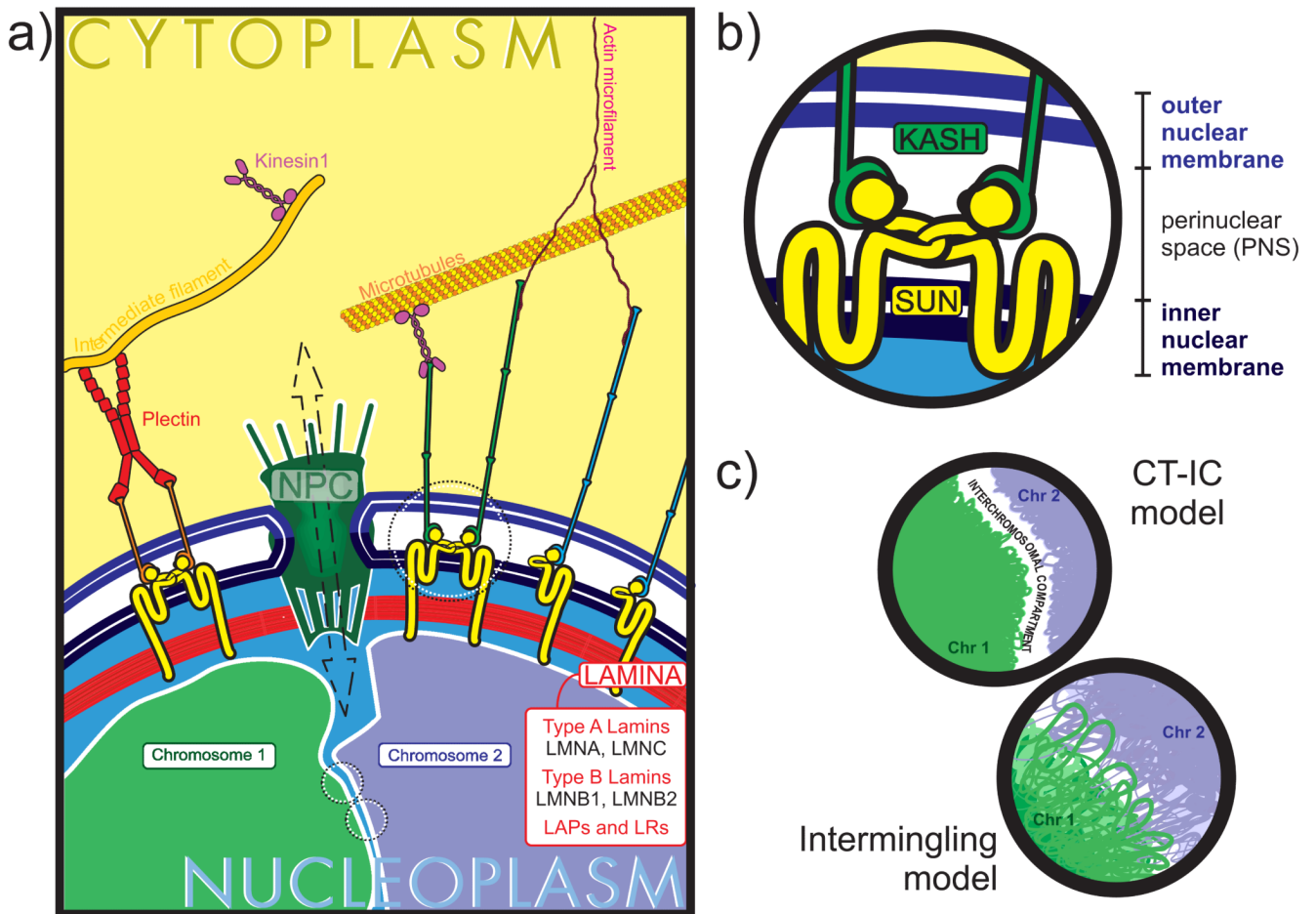


Figure 1.

The archetypal SUN-KASH domain association in the LINC complex. (a) The LINC complex is typified by an association between SUN-domain proteins (yellow) on the inner nuclear membrane and KASH-domain proteins (green, blue, and orange) on the outer nuclear membrane. KASH-domain proteins form a functional link with the networks of cytoplasmic intermediate filaments, microtubules, and actin microfilaments, which compose the cytoskeleton. SUN-domain proteins bind to the nuclear lamina, a network of intermediate filaments composed of varying isoforms of A- and B-type lamins, LAPs, and LRs. The lamina also serves as a tethering point for the genome, with associations reported among various lamins, LAPs, and LRs. (b) KASH domains associate with SUN domains in the perinuclear space, and this association maintains the architecture of the nuclear envelope. SUN proteins can associate promiscuously with KASH proteins and can also form homo- and heterodimers with other SUN proteins. (c) Two competing models explain the 3D organization of CTs in the interphase nucleus. The CT-IC model posits that a largely DNA-free compartment of contiguous spaces between adjacent CTs exists. The second model, known as the intermingling model, maintains that whereas CTs occupy nonrandom spaces in the interphase nucleus, there is a large amount of intermingling of the chromatin between adjacent CTs. Abbreviations: CT, chromosome territory; IC, interchromosomal compartment; KASH, Klarsicht/ANC-1/Syne-1 homology; LAP, lamin-associated protein; LINC, linker of the nucleoskeleton and cytoskeleton; LR, lamin receptor; NPC, Nuclear Pore Complex; SUN, Sad1 and UNC84.

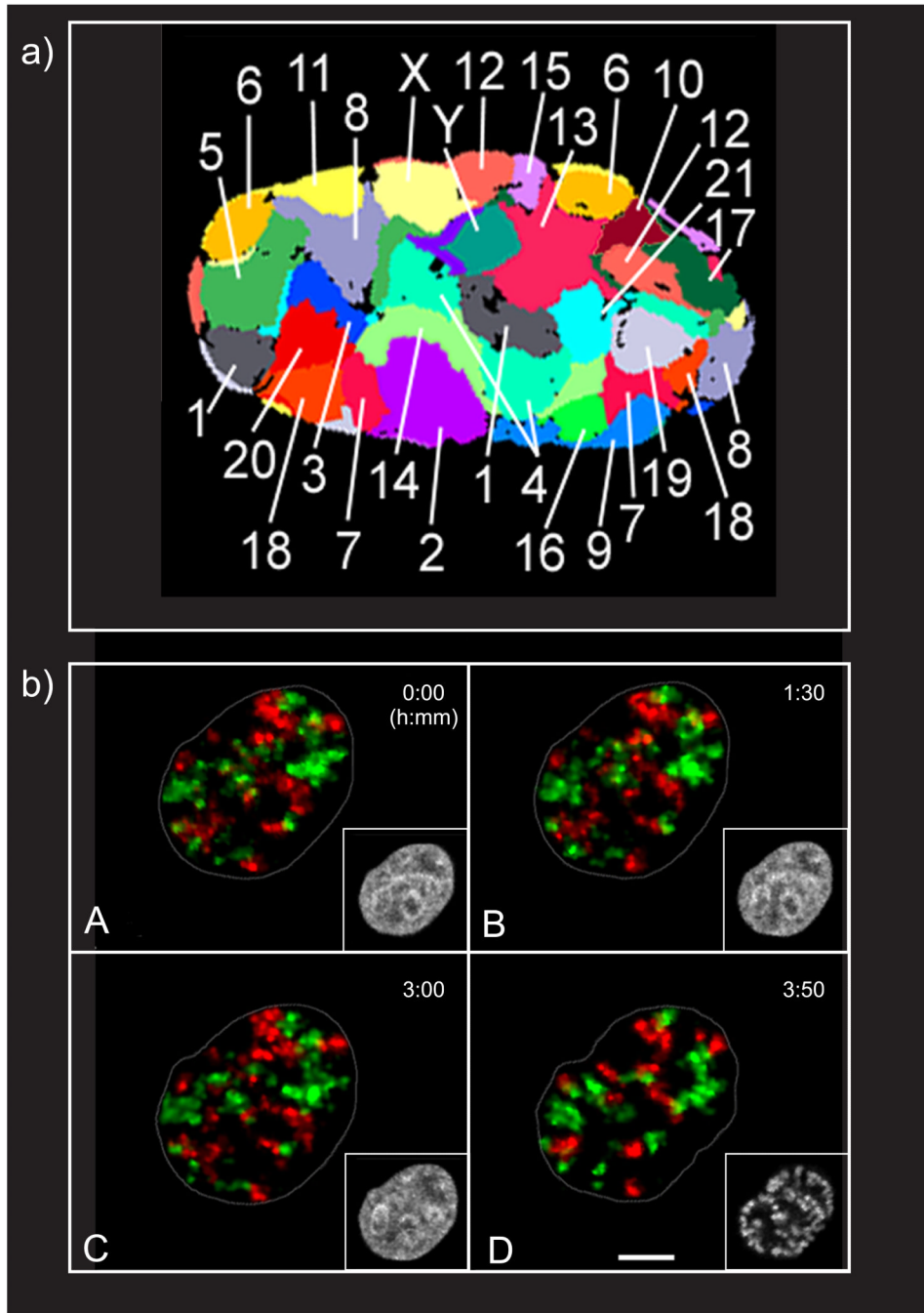
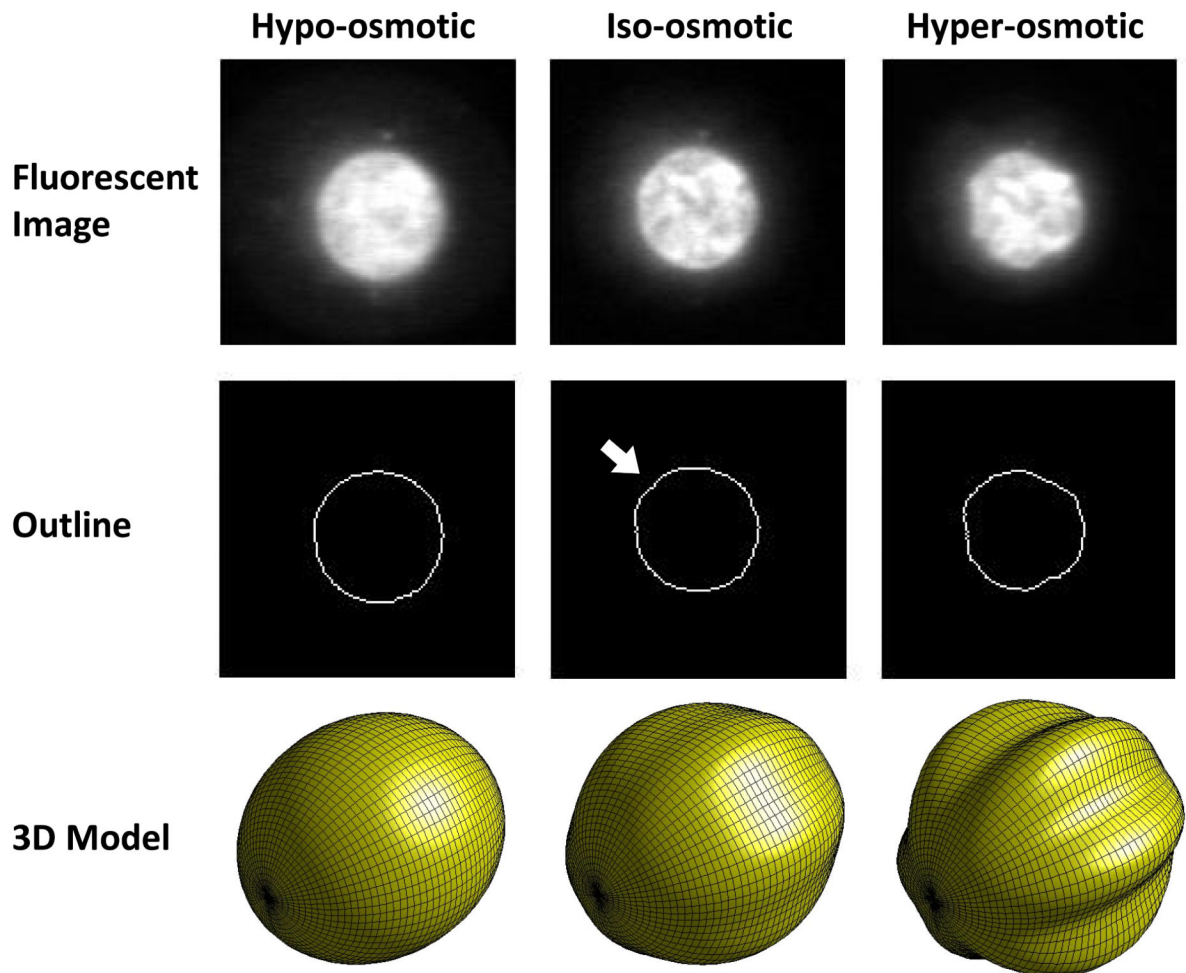


Figure 2. Chromosomes occupy nonrandom placement in the cell nucleus. (a) Flattened 3D fluorescent in-situ hybridization representation of all 24 chromosome territories (CTs) in a human G0 fibroblast nucleus (166). (b) Stability of CT neighborhood during interphase of living HeLa cells. HeLa cells were replication-labeled during S-phase of two consecutive cell cycles (first cycle, Cy3-dTUP, *red*; second cycle, Cy5-dUTP, *green*). (A) Cells were allowed to complete another two cycles before observation was started. (D) After 3 h, 50 min, the cell entered prophase. Frames of maximum intensity projections from light optical serial sections are displayed for the indicated time point. Insets show H2B-GFP signals representing the chromatin (*gray*) in confocal nuclear midsections. Images are corrected for

translational and rotational nuclear movements. Prophase chromosome condensation is a locally confined process with little change to CT arrangements (compare late G2 in subpanel C with early prophase in subpanel D). Scale bar: 5 μm (167). Abbreviations: Cy, cyanine; dUTP, 2'-deoxyuridine 5'-triphosphate; GFP, green fluorescent protein. Adapted from References 166 and 167 with permission.

a

**Figure 3.**

Changes in the nuclear morphology of chondrocytes with osmotic stress. (a) The middle column depicts the iso-osmotic or equilibrium condition (380 mOsm). The osmotic pressurization of the nucleus is barely sufficient to induce mild buckling of the nuclear lamina, as evidenced by the mild undulation in the outline of the nucleus (*white arrow*). The image in the bottom row of the middle column shows a 3D view of a mildly postbuckled spheroid. The left column depicts the hypo-osmotic condition (180 mOsm). The undulation has disappeared, and the outline of the nucleus is now smooth. The right column depicts the hyperosmotic condition (580 mOsm). Now there are pronounced undulations along the entire circumference of the nuclear outline. The image in the bottom row of this column

shows a 3D view of a spheroidal geometry with pronounced longitudinal buckling. These reconstructions are hypothesized to be representative of the nuclear outlines depicted in the middle row. Adapted from Reference 135 with permission. (b) Hyperosmotic challenge on live bovine chondrocytes transfected with H2B-GFP. Chondrocytes were cultured in monolayer initially in an iso-osmotic environment (300 mOsm kg^{-1}) and subjected to hyperosmotic challenge (700 mOsm kg^{-1}) for 15 min, and then brought back to 300 mOsm kg^{-1} for an additional 15 min while being imaged by time-lapse confocal microscopy. The top row shows an average intensity projection in the xy plane. The middle row shows an average intensity projection in the xz plane. The bottom row shows the middle section of the chondrocyte nuclei. Images were thresholded using the iterative self-organizing data algorithm (134; J. Irianto, R.P. Martins & D.A. Lee, unpublished data). Abbreviations: GFP, green fluorescent protein; mOsm, milliosmole.

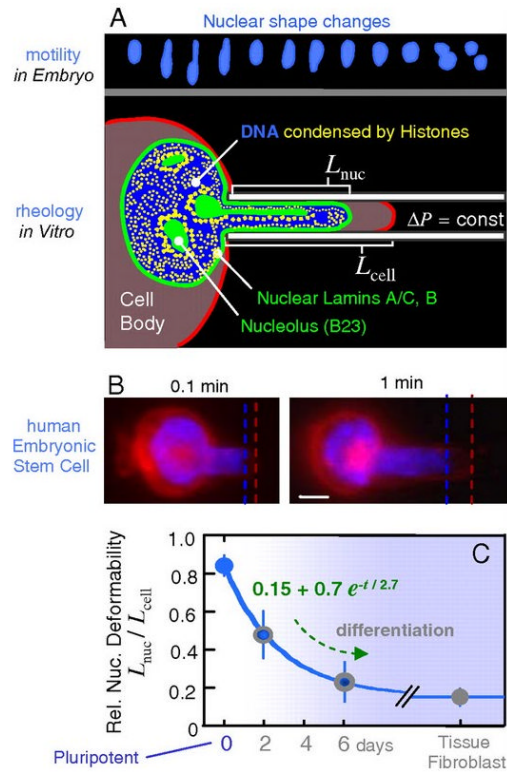


Figure 4. Nuclei of human stem cells are more deformable than nuclei of differentiated cells. (a) Neural progenitor cell nuclei show large deformations during in vivo migration. Micropipette aspiration mimics such distortions. (b) Human embryonic stem cells (ESCs) were aspirated after differentiation in culture, and the ratio of nuclear extension to cytoplasmic extension was measured as L_{nuc}/L_{cell} . A day-zero pluripotent ESC is shown with labeling of nuclear DNA (blue) and the cell membrane (red). Scale bar: 3 μm . (c) As differentiation progresses, ESC nuclei stiffen nearly six-fold relative to cytoplasm, and the decrease in relative compliance fits an exponential decay. Differentiated cells such as embryonic fibroblasts also have a nucleus that is stiffer than the cytoplasm. Reproduced from Reference 142 with permission.

Table 1

Molecular associations between Sun and Nesprin proteins of the LINC complex

Nucleoplasmic partners/function	SUN domain protein	KASH domain protein	Cytoplasmic partners/function
Anchorage between lamina and INM Nuclear positioning (158, 159) Maintenance of perinuclear space Anchorage of telomeres (160, 161)	Sun1 and Sun2	Nesprin 1 and nesprin 2 (multiple variants including GIANT isoforms)	Anchorage between ONM and actin filaments in cytoskeleton Nesprin 2 links nucleus to MTOC and kinesin-1 (162)
		Nesprin 3	Anchorage to intermediate filaments via plectin adaptor protein (60)
Postmeiotic, sperm-specific isoforms (163)	Sun1 γ and Sun3	Nesprin 1 and nesprin 3	Manchette microtubules
Sperm-specific isoform (164)	Spag 4 and Spag 4L/2	Unknown	Unknown
Nuclear positioning	Unknown	Nesprin 4	Connects to kinesin-1 (165)

Abbreviations: INM, inner nuclear membrane; KASH, Klarsicht/ANC-1/Syne-1 homology; LINC, linker of the nucleoskeleton and cytoskeleton; MTOC, microtubule organizing center; ONM, outer nuclear membrane; Sun, Sad1 and UNC84; Spag4, sperm associated antigen