REVIEWS

Regulation of genome organization and gene expression by nuclear mechanotransduction

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Abstract | It is well established that cells sense chemical signals from their local microenvironment and transduce them to the nucleus to regulate gene expression programmes. Although a number of experiments have shown that mechanical cues can also modulate gene expression, the underlying mechanisms are far from clear. Nevertheless, we are now beginning to understand how mechanical cues are transduced to the nucleus and how they influence nuclear mechanics, genome organization and transcription. In particular, recent progress in super-resolution imaging, in genome-wide application of RNA sequencing, chromatin immunoprecipitation and chromosome conformation capture and in theoretical modelling of 3D genome organization enables the exploration of the relationship between cell mechanics, 3D chromatin configurations and transcription, thereby shedding new light on how mechanical forces regulate gene expression.

Transdifferentiation
The process in which a somatic cell is transformed into another type of somatic cell.

Integrins
Cell surface transmembrane receptors involved in mechanosensing.

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In the tissue microenvironment, cells are subjected to a wide range of physical and chemical signals[1,2] (FIG. 1). For example, endothelial cells are exposed to both shear and tensional forces and to a combination of growth factors from the blood flow[3]. By contrast, cells of epithelial and connective tissues experience relatively more tensional forces, either from stretching or from compression, and are exposed to a combination of cytokines and growth factors in order to maintain cellular homeostasis[4]. Lymphocytes experience compressive forces when egressing from or transmigrating between tissues and the bloodstream[5]. Importantly, cells adapt to their microenvironment by fine-tuning their mechanical properties[6]. For example, whereas brain cells are soft, bone cells are stiff. Cells integrate local signals from their microenvironment and express cell type-specific genes to maintain tissue homeostasis. However, when cells are subjected to extreme mechanical deformations, such as strong shear, strain or compression, they may alter their gene expression programmes to counterbalance such stresses, which in extreme cases change cell type and initiate transdifferentiation, possibly causing diseases such as cancer[7–10].

Cellular responses to such diverse signals from their microenvironment crucially depend on accurate sensing mechanisms and the magnitude of the signals[11,12]. A number of cell surface protein assemblies have been characterized that are able to sense physical and chemical signals. For example, integrins in focal adhesion complexes sense the rigidity or geometry of the extracellular matrix[13], stretch-activated receptor assemblies sense the microenvironment and adapt their permeability to various extracellular ions[14], and cadherin assemblies sense mechanical signals at cell–cell junctions and mechanically couple neighbouring cells[15]. In addition, there are numerous other well-characterized receptors, such as G protein-coupled receptors and Notch receptors, that have been shown to respond to both mechanical and biochemical signals in the microenvironment[16].

An underlying principle of most sensing mechanisms is the association of receptor molecules into protein assemblies to enhance signal sensitivity and allow efficient intracellular mechanotransduction. A key intermediate in such receptor-clustering mechanisms is the active reinforcement of the actomyosin machinery at the inner cell membrane[17]. Recently, it was discovered that actomyosin-induced clustering also exposes buried amino acid residues by unfolding junction proteins, thereby facilitating biochemical signal transduction[18].

The perception of mechanical signals from the microenvironment has important functional implications. For example, mesenchymal stem cells sense the underlying rigidity of the extracellular matrix as part of their differentiation into various cell types[19] and were shown to respond to the topographical constraints of their attachment to the extracellular matrix to differentiate into osteogenic or adipogenic lineages[20]. Haematopoietic stem cells in the bone marrow

Focal adhesion complexes
Cell membrane protein complexes that connect the actin cytoskeleton with the extracellular matrix.

Cadherin
A transmembrane receptor that bridges cell–cell junctions.

Actomyosin
Protein complexes comprising actin and myosin; they form contractile units.

sense shear forces generated by the blood flow, thereby enabling them to differentiate into the various blood lineages[21]. In response to local mechanical signals, epithelial cells can undergo an epithelial–mesenchymal transition, which is crucial in establishing early-developmental expression programmes[8]. Importantly, defects in cellular mechanosensory processes have been associated with a number of diseases such as fibrosis, compromised immune response and various types of cancer[8,22,23].

A major missing link in our understanding of cellular responses to mechanical signals is how such signals are transduced to the nucleus and regulate gene expression programmes in the context of the spatial and
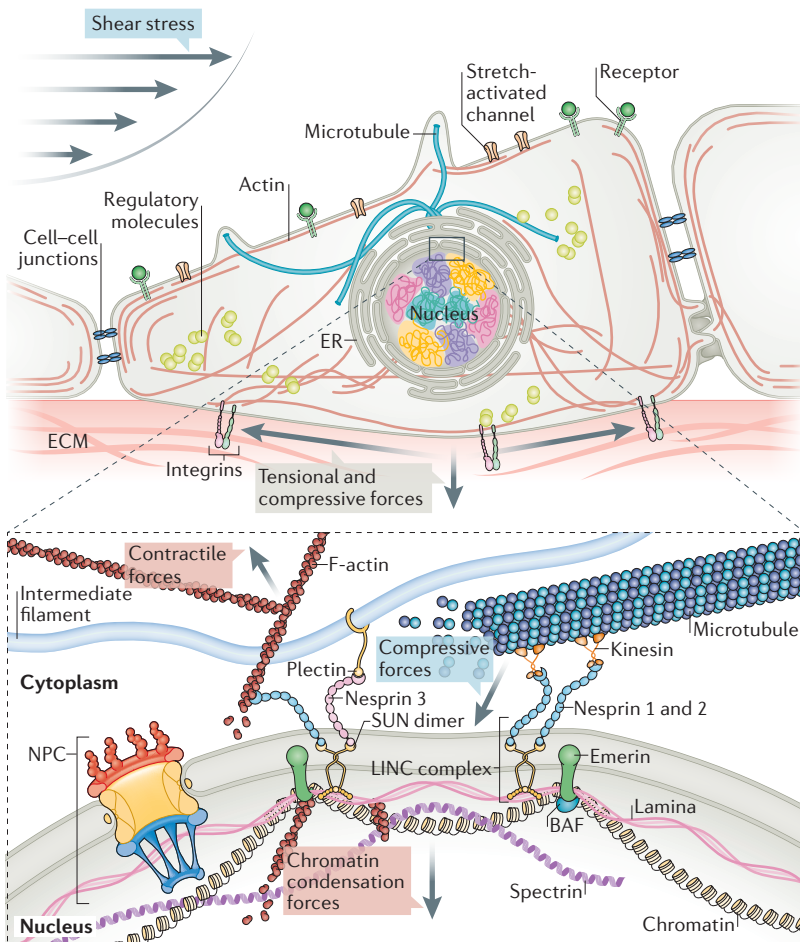

Figure 1 | **Nuclear mechanotransduction.** (Top panel) Cellular microenvironment and mechanosensing. In addition to soluble signals, cells experience shear, tensional and compressive forces in their microenvironment. These signals are sensed through receptors and are transduced to the nucleus through the cytoskeleton networks of actin and microtubules as well as by regulatory molecules. These signals modulate the three-dimensional organization of chromosomes in the nucleus to regulate gene expression programmes. (Bottom panel) Coupling between cytoskeleton filaments and the nucleus through the linker of nucleoskeleton and cytoskeleton (LINC) complex. Nesprins (proteins with Klarsicht–ANC-1–SYNE homology (KASH) domains) on the outer nuclear membrane physically link with actin, microtubules and intermediate filaments. The nesprins are in turn connected to the inner nuclear membrane through dimers of SUN (Sad1p–UNC-84)-domain proteins, which are further linked to the nuclear lamina and chromatin. These cytoskeleton–nucleus links apply differential mechanical forces on the nucleus. Actin exerts contractile forces, whereas microtubules exert compressive forces. BAF, barrier-to-autointegration factor; ECM, extracellular matrix; ER, endoplasmic reticulum; NPC, nuclear pore complex.

temporal organization of the genome[24]. A number of studies in recent years have highlighted the importance of cytoplasmic-to-nuclear shuttling of various transcription factors as well as the nonrandom organization of chromosomes within the nucleus as important regulators of gene expression[25–27]. Signals that are sensed at the cell membrane are transduced through various secondary messengers to activate transcription factors. These transcription factors are then relayed to their target sites within the cell nucleus. However, how mechanical signals activate specific transcription factors is poorly understood, as is how these transcription factors activate gene expression patterns[28]. Understanding these processes would be particularly important for gaining mechanistic insights into how cells elicit distinct expression patterns in response to the same biochemical signal in different cellular mechanical states.

In this Review, we discuss the current understanding of nuclear mechanotransduction processes and the coupling between nuclear mechanical properties and gene expression programmes. We first discuss the activation of nuclear mechanotransduction pathways by local signals and provide an overview of nuclear mechanical properties and how these properties adapt and change in response to cues from the microenvironment. We then discuss the mechanoregulation of 3D nuclear organization and its dynamics, as well as how this is coupled to chromosome organization and gene expression. Finally, we provide a perspective on the crucial role of such nuclear mechanogenomic codes for the maintenance of tissue homeostasis and discuss how alterations in these codes can lead to changes in cell states and cell behaviour, with pathological consequences.

Mechanotransduction to the nucleus

Mechanical and biochemical cues are sensed by specialized membrane proteins and are relayed to the nucleus biochemically and/or physically[24,25,29]. Biochemical signals are transduced either directly from the cell membrane to the nucleus or by activating secondary messengers in the cytosol. The transmission of biochemical signals is facilitated by cytoskeletal remodelling[25,30]. For example, the polymerization state of actin regulates the cytoplasmic-to-nuclear localization of various transcription factors[31]. Mechanotransduction by the YAP and TAZ transcription factors pathway and the function of the actomyosin cytoskeleton in this process are reviewed in REF. 25.

The cytoskeleton networks bridge the cell membrane and the nucleus through the linker of nucleoskeleton and cytoskeleton (LINC) complex, thereby allowing the direct transmission of mechanical signals[32,33]. These cytoskeleton networks are attached to the nuclear membrane and are also directly linked to the adhesive complexes of the extracellular matrix and cell–cell junctions[32]. The best characterized cell membrane–nucleus links are the actin–nesprin links[34–36], defects in which may lead to impaired nuclear mechanotransduction[37,38]. Specifically, nesprins, which are KASH (Klarsicht–ANC-1–SYNE homology)-domain proteins located on the outer nuclear membrane, are LINC components connected to actin

filaments as well as to microtubules and intermediate filaments (FIG. 1). The nesprin proteins are also connected directly to LINC proteins at the inner nuclear membrane — the SUN (Sad1p–UNC-84)-domain proteins — thereby forming a physical bridge between the outer and inner nuclear membranes. The SUN-domain proteins are in turn connected to the nuclear lamina and to chromatin through a number of adaptor proteins, thereby providing a direct mode of physical signal transmission from the cell membrane into the nucleus[32–39].

Mechanical cues sensed at the plasma membrane are directly propagated through the cytoskeleton networks to the nucleus[40]. For example, studies using magnetic twisting cytometry showed that application of local stresses on the cell surface induced the transcription of a GFP-tagged transgene[41], thereby demonstrating the importance of physical links for mechanotransduction. Consistent with this finding, fibroblasts plated on arrays of micropillars and stably expressing histone H2B–EGFP show a strong correlation between pillar deflection induced by traction forces and heterochromatin movement within the nucleus, thereby revealing a direct visco-elastic coupling between the cell membrane and the nucleus[42]. In addition, in isolated HeLa cell nuclei, forces applied by magnetic beads on nesprin 1 at the outer nuclear membrane resulted in the phosphorylation of emerin, which resides at the inner nuclear membrane[43]. Furthermore, in HeLa cells, the DNA repair kinase ATR is sequestered to the nuclear membrane and activated by osmotic stress and mechanical stretching[44]. Osmotic swelling of cells and their nuclei, which results in increased membrane tension, also promotes calcium-dependent activation of enzymes involved in eicosanoid biosynthesis[45]. Recent experiments have also shown that cells passing through narrow channels or cells aspirated in micropipettes undergo nuclear deformation, DNA damage and the activation of DNA repair pathways[46–48]. Taken together, these observations reveal a novel link between gene expression, genomic stability and mechanical signals.

Mechanical stretching of cells or changes to their geometry using micropatterning techniques have also been shown to lead to the remodelling of cytoskeletal organization[40–51]. This remodelling includes, for example, the calcium-mediated remodelling of actin stress fibres for the transient reorganization of perinuclear actin[52]. Notably, inverted formins, such as inverted formin 2, that are localized on the nuclear membrane and the endoplasmic reticulum, are activated by increased concentrations of G-actin resulting from force-induced actin depolymerization[53]. The activation of inverted formin 2 results in the transient polymerization of the perinuclear actin ring, potentially protecting the genome from large-scale mechanical forces experienced by cells. Furthermore, cell geometry-induced actin remodelling has been shown to result in chromatin condensation, which can lead to transcriptional quiescence[54,55]. Although cytoskeleton remodelling can support biochemical and direct physical transmission of signals to the cell nucleus, it also impinges on the mechanical properties of the nucleus as discussed in the following section.

Nuclear mechanics

Another important aspect of nuclear mechanobiology is the adaptability of mechanical properties of the nucleus itself to regulate gene expression. The mechanical properties of the nucleus are determined by the interplay of cytoskeleton–nucleus links, by the integrity and composition of the nuclear lamina and by the degree of DNA packaging into chromatin[32,56–58] (FIG. 1). For example, fibroblasts plated on micropatterned substrates of polarized shapes have stronger actin-mediated links to the cell nucleus than fibroblasts plated on isotropic shapes[51]. Similarly, fibroblasts growing on rigid substrates have stronger actin-mediated links to the nucleus than those growing on soft substrates[58]. These links are part of an elaborate architecture of perinuclear actin, which exerts a mechanical strain on the nucleus and determines its size and shape[59]. On polarized shapes or rigid substrates, the apical actin stress fibres compress the nucleus into a flat ellipsoid, whereas on isotropic or soft substrates, the more relaxed depolymerized actin structures result in the loss of mechanical tension and hence a spherical nucleus. The contractile forces applied on the nucleus by the actin cytoskeleton are counterbalanced by microtubules that exert compressive forces on the nucleus, as was revealed by RNAi screens combined with specific ablation of actin or microtubules using pharmacological inhibitors[60,61]. Microtubules and intermediate filaments can also be reorganized to modulate the nuclear morphology and its deformability. Importantly, the fine balance between the contractile and compressive forces exerted by actin and microtubules, respectively, determines the nuclear morphology and thereby affects gene expression as discussed below.

The capacity for nucleus deformation critically depends on its stiffness. A number of recent studies have shown that the levels of nuclear lamins (lamin A/C and lamin B), in particular lamin A/C proteins, scale with nuclear stiffness[62–64]. Nuclear lamins are intermediate filaments that provide structural integrity to the cell nucleus. For example, stem cells have low levels of lamin A/C and are soft, whereas somatic cells have higher levels of lamin A/C, resulting in tissue-specific nuclear stiffness. Lamin A/C proteins directly bind to the inner nuclear membrane proteins, whereas lamin B is an integral component of higher-order chromatin structure[63]. Lamin A/C proteins are linked to cytoskeletal filaments through the LINC proteins of the outer nuclear membrane, thereby contributing to an elaborate protein meshwork that determines the stiffness of the nucleus. Importantly, the dynamic structural conformation of lamin A/C is tension sensitive and affects chromatin anchoring to the nuclear envelope and 3D chromatin conformation[62,65]. Interestingly, recent studies have revealed that lamin A/C phosphorylation and turnover are determined by the topography and rigidity of the extracellular matrix. For example, cells grown on polarized or rigid substrates have higher levels of lamin A/C than their counterparts grown on isotropic or soft substrates[64].

The third determinant of nuclear mechanical properties is the degree of DNA packaging into chromatin,

Junction proteins
Proteins that bridge the cytoskeleton of two neighbouring cells through the cell–cell junction.

Magnetic twisting cytometry
A technique for applying precise forces to single cells.

Visco-elastic coupling
The propensity of materials to exhibit viscous and elastic responses when deformed.

Inverted formins
Actin-nucleating proteins located on the endoplasmic reticulum and other cellular organelles.

G-actin
The globular form of monomeric actin.

Box 1 | The functional organization of the genome

In recent years, the 3D organization of chromatin in the nucleus has emerged as being crucial for genome regulation[26,134–136]. The use of fluorescence *in situ* hybridization (FISH) has revealed that each chromosome occupies a distinct nuclear territory and that the 3D positioning of chromosomes correlates with chromosome size and gene density[137] (see the figure). Larger and more gene-poor chromosomes tend to be localized at the periphery, whereas smaller and more gene-dense chromosomes tend to be localized in the interior of the nucleus[138]. In addition, the physical distance between chromosomes and the degree of chromosome intermingling correlate with chromosomal gene expression levels, that is, chromosomes with similar expression levels tend to be in greater physical proximity[139]. Such transcription-dependent nonrandom positioning of chromosomes is conserved within a cell type[96,97,128]. Interestingly, regions of chromosome intermingling are enriched with active RNA polymerase II and various transcription factors. These regions are collectively referred to as transcription factories[140] (see the figure).

In addition to FISH, chromosome conformation capture (3C) and its derivative methods (4C, 5C and Hi-C) have been designed to probe the 3D organization of the genome, measuring the genome-wide contact frequencies averaged over a population of cells[141–144]. DNA associates with a number of histone and non-histone proteins, and the resulting chromatin fibre is organized into loops (for example, by forming promoter–enhancer interactions). This fibre is further condensed into domains of a few hundred kilobases in length known as topologically associated domains (TADs), which are internally enriched in *cis* interactions[135]. In turn, TADs are organized into transcriptionally active and inactive (for example, heterochromatin) compartments. Some of the heterochromatin regions are linked to the inner nuclear membrane at lamin-associated domains (LADs; see the figure). High-resolution analysis of chromatin interactions within TADs revealed the presence of sites of constitutively bound CTCF (CCCTC-binding factor) that facilitate chromatin looping interactions[145]. In addition, analysis of interchromosomal interactions determined that regions on neighbouring chromosome territories may also loop out and intermingle with each other in a transcription-dependent manner. Collectively, these studies indicate that interchromosomal regions may harbour co-regulated gene clusters (see the figure).
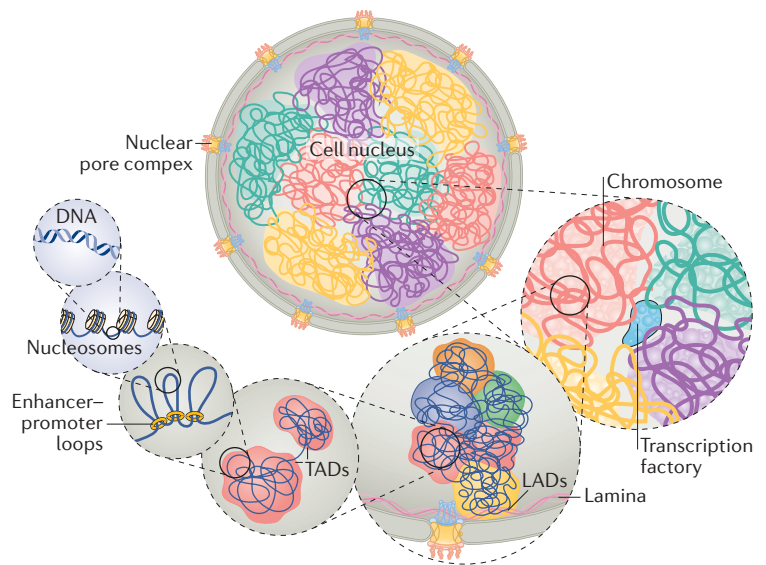
A number of recent studies have correlated the positions of various post-translational modifications, transcription factor binding sites, enhancer–promoter loops and RNA polymerase II occupancy with Hi-C data in order to gain insights into the spatial dimension of gene regulation[106–108]. Chromosome organization models have been introduced to address the mechanical coupling between nuclear morphology and gene expression[146]. In these models, the spatial arrangement of chromosomes is viewed as a configuration of ellipsoids (the chromosome territories) packed into an ellipsoid-shaped container (the nucleus). The shape of the container is defined by mechanical constraints, and cell type-specific configurations are determined by solving an optimization problem, where the pairwise overlap between two chromosomes is penalized on the basis of their difference in gene expression levels. The solutions to this optimization problem are configurations that link nuclear morphology with chromosome organization and gene expression[97,146]. Recent progress in super-resolution imaging, optogenetics, CRISPR–Cas-mediated chromatin labelling and multiplexed Hi-C methods will be valuable for understanding the functional organization of chromosomes[147–151].

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which is crucial for regulating gene expression. The metre-long DNA polymer is wrapped around histones with the assistance of non-histone proteins[66]. As the typical size of a eukaryotic nucleus falls in the range of 10–50 microns, depending on cell type, the chromatin exerts an outward entropic pressure onto the nuclear envelope[67]. This pressure is counterbalanced by a number of post-translational modifications on histone tails that facilitate chromatin condensation[68]. Depending on the type of post-translational modification, such as acetylation or methylation, the DNA is differentially organized into more open regions and more condensed regions (for example, heterochromatin). Some condensed chromatin regions, such as the lamin-associated domains (BOX 1), associate with the nuclear lamins and the inner nuclear membrane through specific adaptor proteins, such as lamin-binding receptors[69]. Thus, the level of chromatin condensation determines the size of the nucleus, as well as its mechanical properties[70,71], through its coupling with the nuclear envelope and the cytoskeleton.

To summarize, the nuclear mechanical properties[72] are dictated by the composite structure of chromatin, nuclear lamina and cytoskeletal filaments. For example, when cells are attached to an isotropic or soft substrate, the nucleus is relaxed owing to a combination of loss of actomyosin contractility, increased compression of the nucleus through microtubules, reduction of lamin A/C levels and increased chromatin condensation.

Nuclear and chromatin dynamics

In addition to tuning the level of nuclear stiffness, recent experiments have revealed that the composite structure of cytoskeleton–lamina–chromatin also determines nucleus and chromatin dynamics, which are crucial for regulating gene expression. In particular, by using time-lapse imaging, the topography of the extracellular matrix was shown to control the translational and rotational movements as well as the volume fluctuations of the nucleus[73–75]. For example, when fibroblasts are attached to polarized or rigid substrates, the position of the nucleus is stable and secured by the apical actin

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Entropic pressure
Forces generated by the intrinsic thermodynamic tendency to increase entropy.

stress fibres. On the other hand, loss of matrix attachment through the inhibition of myosin phosphorylation results in increased translational motility of the nucleus owing to forces exerted by actomyosin contractility[73]. In addition to increased nuclear translational motility, the loss of cell–matrix interactions results in large-scale actomyosin contractile flows that lead to the rotation of the nucleus[74]. Similarly, loss of nuclear lamin B proteins also results in nuclear rotation[76]. Furthermore, actomyosin contractile forces lead to large-scale deformations of the nucleus. Time-lapse imaging of the nucleus revealed that the fluctuations in volume or projected area of the nucleus are regulated both by actomyosin contractility and by nuclear stiffness, which in turn is determined by lamin A/C levels[75].

In addition to increased nuclear motility, cells plated on isotropic or soft substrates show elevated chromatin motility in the nucleus. Visualization of chromatin dynamics in cells stably expressing H2B–EGFP suggests that reduced contacts between the cell and the extracellular matrix result in the detachment of some of the chromatin from the nuclear membrane, leading to increased telomere and heterochromatin motility, for example[75]. In addition to increased global chromatin dynamics, recent experiments using fluorescence recovery after photobleaching also revealed increased turnover of chromatin binding proteins[77]. An RNAi screen showed that depletion of focal adhesion proteins and actin-crosslinking proteins increased not only nuclear motility but also chromatin motility[61]. Such alterations in chromatin dynamics are consequences not only of the loss of physical connections between the extracellular matrix or cell–cell junctions and the nucleus but also of nuclear import of chromatin modifiers such as histone deacetylase 3 (HDAC3)[78]. Collectively, these results suggest that mechanical forces modulate nuclear and chromatin dynamics through physical links as well as biochemical pathways, which in turn tune histone post-translational modification and chromatin structure and lead to differential accessibility of transcription factors to gene-regulatory sites on DNA.

Consistent with these observations, stem cells, which partially lack the cytoskeleton–lamina–chromatin structure, have a hyperdynamic nuclear and chromatin organization[79–81]. This was revealed by experiments of micropipette aspiration of stem cell nuclei, in which stem cell nuclei deformed to a greater extent than differentiated cells and the degree of nuclear deformation decreased as cells progressed through differentiation[79]. In addition, histone turnover experiments using fluorescence recovery after photobleaching revealed that the chromatin structure is highly dynamic in stem cells owing to hyperacetylation of histones[80,81]. Fluorescence anisotropy imaging revealed that the chromatin organization exhibited a more fluid state in stem cells than in differentiated cells[82]. Furthermore, lamin A/C protein levels were found to be low in stem cells and to increase with differentiation according to tissue stiffness[62]. More recently, time-lapse imaging during stem cell differentiation revealed a progressive stabilization of the nuclear and chromatin organization and

the emergence of cellular mechanosensitivity, which is required to adapt to various tissue microenvironments[83,84]. Such genome modulation by the mechanical properties of the tissue is crucial for establishing the cell type-specific organization of chromosomes and the accessibility of regulatory sites to transcription factors, as discussed next.

Mechanoregulation of gene expression

Mechanical and biochemical signals that are sensed at the cell membrane can result in the activation of transcription factors[85–92], which are then recruited to their target sites to activate cell type-specific gene expression programmes. For example, cells have been found to activate different genes when they are subjected to shear, compression or stretch[3,4]. In addition, plating cells on different surface topographies (polarized versus isotropic) changed the shape of their nuclei, which resulted in the activation of different gene expression programmes[78]. Furthermore, cells plated on substrates with different topographies and rigidities can exhibit distinct behaviours in terms of proliferation, differentiation and apoptosis[7,19,20]. An earlier study showed that systematic tuning of the contact area between the cell and extracellular matrix resulted in altered expression of the extracellular matrix protein collagen[93]. These observations suggest three possible, non-exclusive mechanisms by which the microenvironment can regulate gene expression: control of the nuclear import of different transcription factors, alteration of 3D nuclear organization and chromosome intermingling (BOX 1) and spatiotemporal mechanoregulation of gene clustering.

Mechanoregulation of transcription factors. To analyse the three possible mechanisms, we discuss the example of cells plated on a surface with polarized versus isotropic geometry or equivalently on a rigid versus soft substrate. Fibroblasts plated on polarized geometries express relatively more cytoskeleton and matrix genes, whereas the same cells plated on isotropic geometries express relatively more cell–cell junctions and cell cycle genes[78]. This finding is brought about by, among other factors, the activity of the serum response pathway in cells growing on polarized geometries and by the nuclear factor-κB (NF-κB) pathway in cells growing on isotropic geometries. Such modular switching in the activation of gene expression programmes between the two cellular mechanical states is induced by alterations in actomyosin contractility that result in the nuclear import of the transcription factor of each pathway, namely, myocardin-related transcription factor (MRTF; also known as MKL/myocardin-like protein) and p65, respectively.

An important example of mechanoregulation of transcription factors is the crosstalk between MRTF, NF-κB and HDAC3 (FIG. 2). MRTF is a transcription cofactor bound to G-actin[86]. Increasing cell polarization results in the polymerization of actin[49], which requires G-actin to be recruited to F-actin stress fibres, thereby releasing MRTF to the nucleus, where it binds to serum response factor (SRF). By contrast, reducing

cell polarization results in the depolymerization of actin and an increase in G-actin concentration, thereby leading to the sequestration of MRTF from the nucleus to the cytoplasm. Interestingly, recent studies have revealed the existence of crosstalk between the NF-κB and serum response pathways through alterations in actomyosin contractility[94]. Namely, decreased actin polymerization results in the translocation of the NF-κB transcription factor p65 to the nucleus, while MRTF is exported to the cytoplasm (FIG. 2). Notably, the reduction in actomyosin contractility due to actin depolymerization also results in the shuttling of HDAC3 to the nucleus, which results in increased chromatin condensation[78,95]. This is an important example of the mechanical control of chromatin condensation and gene expression through the differential nuclear localization of transcription factors and chromatin modifiers. We propose that such mechano-dependent crosstalk is a generic mechanism to regulate context-dependent cytoplasm-to-nucleus shuttling of various transcription factors.

Mechanoregulation of 3D nuclear organization. As discussed above, changes in actomyosin contractility also result in major alterations to nuclear morphology, suggesting that the 3D nuclear organization (BOX 1) has an important role in modulating gene expression programmes. Recent studies using fluorescence *in situ* hybridization (FISH) in interphase cells plated on micropatterned substrates have revealed that the relative positions of chromosomes in the nucleus are dependent on the mechanical state of the nucleus[96,97]. For example, fibroblasts attached to polarized substrates have a flat, ellipsoidal nucleus and preferentially orient their chromosomes along the mechanical axis of the nucleus that is parallel to the nuclear attachment (FIG. 3). The same cells attached to isotropic substrates have a more spherical nucleus and also preferentially align their chromosomes with the mechanical axis of the nucleus, but owing to the altered shape of the nucleus, the axis is perpendicular to their attachment. Interestingly, the chromosomes most aligned (most in parallel) with the mechanical axis are the most transcriptionally active in the respective topographies[97]. The differential repositioning of chromosomes in fibroblasts plated on isotropic shapes is due to the downregulation of lamin A/C, which leads to a reduction in the interaction between chromosomes and the inner nuclear membrane. In addition, downregulation of lamin A/C results in increased nuclear dynamics, thereby enabling relative displacement of chromosomes within the nucleus, leading to the formation of new chromosome surroundings and interactions (neighbourhoods)[97].

The alterations in the alignment and neighbourhoods of chromosomes change the domains that intermingle between chromosomes. FISH experiments revealed that the chromosomes that are most aligned with the mechanical axis of their nucleus show a higher degree of intermingling. Importantly, these intermingling regions were found to be enriched with active RNA polymerase II (Pol II), indicating that the
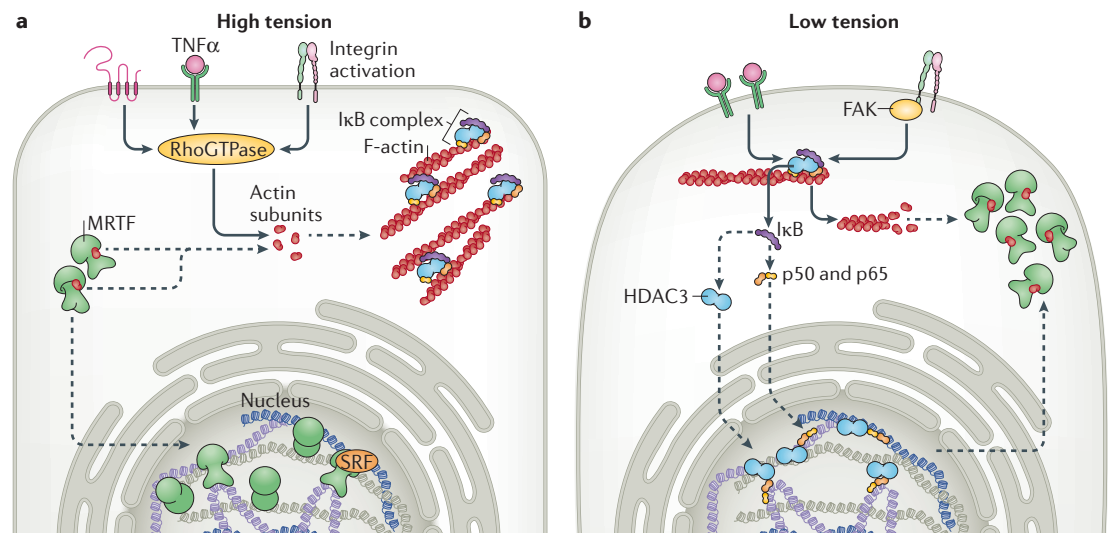
Figure 2 | **Crosstalk between the serum response pathway, the NF-κB pathway and global chromatin remodelling.** **a** | Cells grown on polarized or rigid substrates are subjected to increased tension and undergo actin polymerization, which results in the disassociation of the G-actin–myocardin-related transcription factor (MRTF) complex. Subsequently, MRTF localizes to the nucleus to activate MRTF–serum response factor (SRF) target genes. Concomitantly, the increased polymerization of actin leads to cytosolic localization of the NF-κB factors p50 and p65 and of histone deacetylase 3 (HDAC3), resulting in the downregulation of NF-κB target genes. **b** | Cells grown on isotropic or soft substrates are subjected to lower levels of tension and undergo actin depolymerization. MRTF is localized more in the cytosol, whereas NF-κB and HDAC3 are localized more in the nucleus. Furthermore, stimulating these cells with cytokines, such as tumour necrosis factor a (TNFα), results in the nuclear localization of the TNFα downstream target NF-κB in both high-tension and low-tension regimes. However, the cells in these two tension regimes express distinct groups of genes, potentially due to distinct chromosome organization. Dashed arrows indicate reversible reactions and pathways. FAK, focal adhesion kinase; IκB, inhibitor of NF-κB.
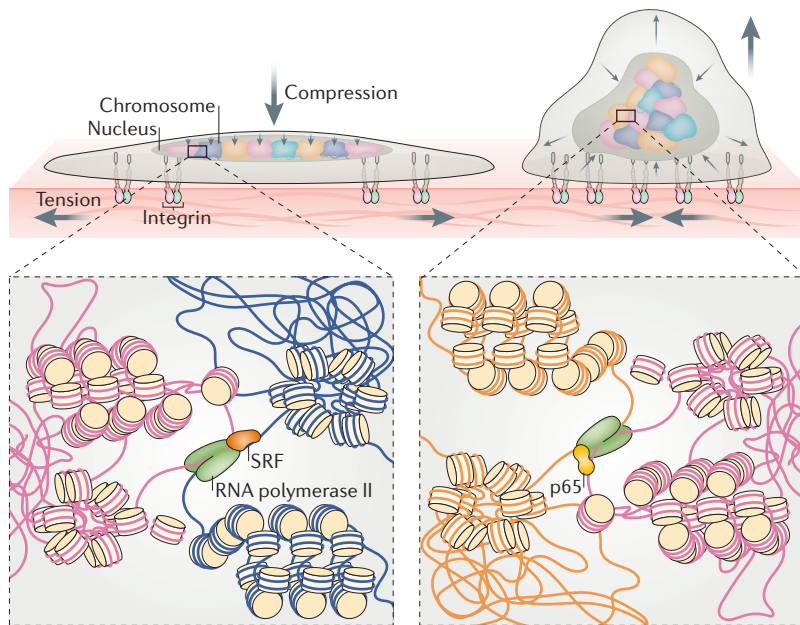
Figure 3 | **The modulation of chromosome intermingling and gene neighbourhoods is induced by cell mechanical constraints.** The mechanical state of a cell modulates nuclear morphology and with it the three-dimensional organization of chromosomes, thereby establishing specific patterns of chromosome intermingling. Such intermingling regions harbour different genes that are spatially clustered by their corresponding transcription factors, such as serum response factor (SRF; left panel) or p65 (right panel), and associate with active RNA polymerase II. This suggests that the spatial clustering and expression of target genes of particular transcription factors are optimized for the mechanical state of a cell.

different gene expression patterns may arise from spatial reorganization of chromosomes (FIG. 3). Consistent with this hypothesis, recent studies have shown that the intermingling regions in the two substrate topographies were also enriched with their respective transcription factors SRF and p65 (REFS 96,97). Furthermore, immunostaining of the transcription factors bound to open chromatin spreads, and subsequent super-resolution imaging of these complexes revealed the existence of clusters of contacts between relatively small chromatin regions[98]. Specifically, in cells attached to polarized substrates, chromatin contacts were enriched with SRF and active Pol II, whereas the same cells on isotropic substrates contained chromatin contacts enriched with p65 and active Pol II. Collectively, these observations demonstrated for the first time that cell mechanical constraints not only alter the nuclear morphology by modulating actomyosin contractility and the shuttling of transcription factors but also modulate the spatial organization of chromosomes in the nucleus and the degree of their intermingling, thereby resulting in differential activation of gene expression programmes.

We suggest that this is a general phenomenon, given that most mechanical and biochemical signals not only activate specific transcription factors but also alter nuclear and chromosomal organization. Our hypothesis is that during differentiation, the spatial organization of chromosomes in the nucleus is optimized to activate cell-type-specific gene expression programmes[96]. This could

be one of the main mechanisms by which mesenchymal stem cells plated on different matrix rigidities differentiate into different cell types[19]. Similarly, different spatial configurations of chromosome arrangements and their intermingling imposed by different cell geometries in mesenchymal stem cells could enable the activation of osteogenic or adipogenic gene expression programmes[20]. In line with this hypothesis, we suggest that large-scale mechanical perturbations such as shear, stretch or compression lead to distinct patterns of chromosome intermingling and result in the activation of differential gene expression programmes.

Mechanoregulation of spatial gene clusters. A number of recent studies have revealed that not only the relative positioning of chromosomes in the nucleus but also the spatial organization of genes is important for regulating gene expression (FIG. 3). For example, using FISH, active genes were found to be spatially clustered, which was necessary for their expression[99]. More recently, a combination of chromatin conformation capture (3C) techniques with chromatin immunoprecipitation followed by sequencing (ChIP–seq) analysis also revealed spatial clustering of functional genes, such as the Hox gene cluster[100]. In addition, a recent study using 3C assays revealed that stimulation of fibroblasts by tumour necrosis factor α (TNFα) resulted in the spatial clustering of TNFα target genes[101]. Furthermore, repeated stimulation of cells revealed that the spatial repositioning of genes upon stimulation was memorized[102]. To investigate the necessity of chromatin interactions for the co-regulation of gene clusters, a single-cell strategy using transcription activator-like effector nucleases to perturb gene neighbourhoods revealed that the expression of NF-κB-regulated genes was abrogated upon contact disruption and was recovered once the contact disruption was repaired[103]. Importantly, in polarized fibroblasts, co-labelling active Pol II and SRF with SRF target genes such as *ZYX* revealed that functional gene clusters were localized within regions of chromosome intermingling[96,97]. These findings suggest that the spatial repositioning of chromosomes and their intermingling link large-scale mechanical alterations of the nucleus to small-scale clustering of co-regulated genes.

Recent studies have also revealed that the temporal order of spatial clustering is crucial for gene co-regulation[103,104]. A theoretical analysis suggested that such a rigid regulatory code, which takes into account the temporal order of spatial clustering, is necessary in order to obtain the various gene expression programmes of different cell types from a limited number of genes and transcription factors[105]. Current gene expression models assume that spatially clustered genes are colocalized with transcription factors, chromatin modifiers and transcription machinery to enable efficient gene expression[106–108]. A hypothesis for how genes are physically brought together may involve the recruitment of transcription factors to their different target sites and their subsequent clustering through engagement with the dynamic transcription machinery. The dynamic assembly of Pol II complexes was recently observed by
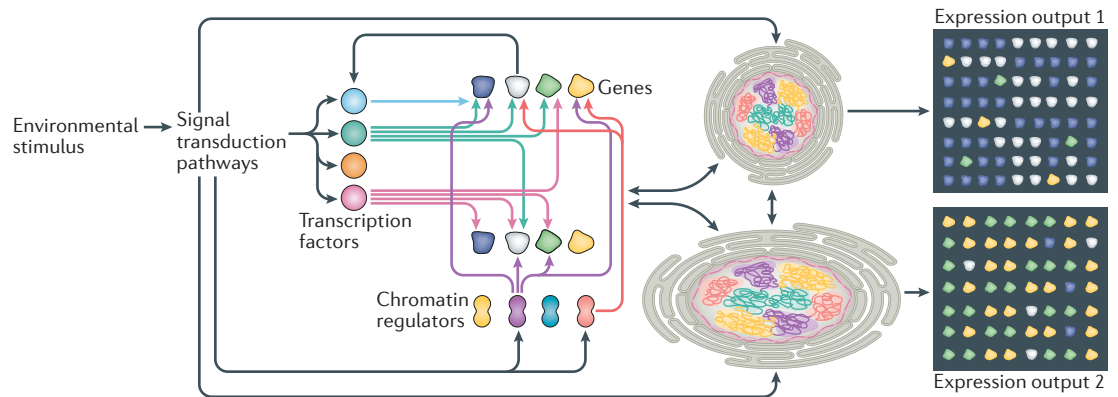
Figure 4 | **The modularity in chromosome organization and gene expression depends on nuclear mechanical state.** We hypothesize that mechanosensing of the extracellular signals from the microenvironment results in both activation of specific transcription factors and modulation of the cytoskeleton–nucleus links, leading to the arrangement of particular chromosome and gene neighbourhoods. The particular chromatin spatial configurations and post-translational modifications are important for guiding transcription factors to their target genes and obtaining optimal transcriptional outputs to maintain cellular homeostasis. We suggest that the loss of such spatial chromatin configurations can lead to transdifferentiation and the onset of various diseases.

super-resolution microscopy[109]. Such spatial and temporal rearrangements of genes require active mechanical forces that can be triggered by actomyosin contractility, subtle alterations in nuclear dynamics or the presence of nuclear actin and myosin in the nucleus[110,111].

We propose that the integration and translation of biochemical signals into different gene expression programmes is enabled by different cellular and thus nuclear mechanical states (FIG. 4). Consistent with this idea, a recent study identified an important link between cell mechanics and cytokine-dependent gene expression: the stimulation by TNFα of fibroblasts plated on polarized versus isotropic substrates resulted in distinct NF-κB-dependent expression patterns, although the NF-κB proteins were similarly localized in the cell nucleus[95,112]. Such experiments provide preliminary evidence that the spatial organization of the genome is optimized for cell type-specific transcription mediated by a diverse set of mechanical and/or biochemical signals.

Mechanogenomics and diseases. Alterations in the microenvironment lead to distinct transcriptional outcomes, and their misregulation can result in the loss of cellular homeostasis. Often, transcription misregulation arises either owing to inappropriate nuclear mechanotransduction or changes in the spatial configuration of chromosomes[45,113]. For example, the epithelial–mesenchymal transition is determined by modular switching in transcription programmes, and its misregulation can lead to oncogenesis[8]. In addition, defects in cytoskeletal regulation of the Hippo pathway can lead to altered organ sizes[114]. Furthermore, defects in NF-κB signalling can compromise immune responses[115].

In addition to inducing defective mechanosignalling, impaired cell–extracellular matrix or cell–cell contacts also result in the disruption of cytoskeleton–nucleus interactions, thereby leading to impaired nuclear morphology[116,117]. For example, in progeria, defective cytoskeleton–nucleus links and mutations in lamin A/C

correlate with abnormal nuclear morphology[118]. In Alzheimer disease, such defective links correlate with altered polymerization of nuclear lamin and abnormal nuclear morphology[119]. Furthermore, various types of cancer show both abnormal nuclear morphology and abnormal chromatin condensation[120]. These alterations in nuclear morphology in various diseases result in the mechanical reorganization of chromosome and gene neighbourhoods. For example, proto-oncogenes are spatially clustered and co-regulated during early stages of tumour development[121]. In addition, chromosome translocations arising from DNA strand breaks[122] are typical of advanced-stage cancer cells. Because cytoskeleton–nucleus links and nuclear morphology are considerably altered in cancer cells, these changes could lead to rearrangement in chromosome positions.

We hypothesize that the mechanical regulation of the nucleus[123–126] changes chromosome structure and modulates the interactions between chromosomes and the nuclear envelope and between genes in intermingling chromosomes[118–128]. These processes could contribute to the onset of various diseases, and such alterations in nuclear morphometrics could be used as physical biomarkers in disease diagnostics[129]. In this regard, a number of recent studies combined high-resolution imaging with machine-learning algorithms to provide preliminary yet promising prognostic tools based on altered nuclear morphometric features[130–132].

Conclusions and future perspective

Mechanical cues from the microenvironment are integrated into gene-regulatory processes through the control of the spatial organization of chromosomes and genes. Recent experiments coupled with theoretical models of chromosome structure (BOX 1) support the notion that the coupling between nuclear mechanotransduction and the spatial organization of the genome has a major role in determining cell type-specific gene expression (FIG. 4).

Progeria
A genetic disorder of premature ageing.

To gain insights into the causal relationship between the spatial organization of the genome and how mechanical signals affect gene expression, a number of questions still need to be addressed. From an experimental perspective, there is a need for major advances in combining super-resolution microscopy with correlated electron microscopy to deduce more precise spatial localization of regulatory factors and gene clusters[133]. This will require the development of innovative labelling and fixation strategies. Furthermore, single-cell resolution of chromosome contact maps and their dynamics could provide better insights into the coupling between nuclear mechanotransduction and differential gene expression programmes. From the theoretical side, there is a need to integrate chromosome structure models with models of signalling and regulatory networks. This would require integration of more local information about chromatin structure at the interface between chromosome-intermingling regions in order to understand how chromosome-intermingling regions are connected to defined spatial clusters of genes and how target genes are accessed by specific transcription factors that interact in a complex biochemical network.

Importantly, each cell type appears to have its own nonrandom arrangement of chromosomes, their intermingling and gene neighbourhoods. In this context, the spatial arrangement of chromosomes and genes provides a paradigm shift in our understanding of how cells could potentially integrate cues from the microenvironment to regulate cell behaviour. This understanding is particularly relevant to explain how the same cue produces different gene expression outcomes in different cell types or in cells subjected to different mechanical constraints. This raises the possibility that the spatiotemporal arrangement of chromosomes could be a major determinant of gene expression programmes and hence suggests the existence of a mechanogenomic code, which may help explain how cellular differentiation as well as reprogramming is controlled by a combination of mechanical and biochemical cues. We conclude that a quantitative understanding of the links between nuclear mechanotransduction and genome architecture is essential to our understanding of cellular homeostasis and pathogenesis and could enable the development of improved diagnostics and novel strategies for early therapeutic interventions.

Finally, we propose the following immediate and outstanding questions and future directions. First, it will be important to develop a better understanding of how microenvironment sensing activates specific transcription factors and translocates them to the cell nucleus. For example, it will be important to establish whether there are any general principles for activating transcription factors by mechanical cues and whether these depend on subcellular localization. It would also be important to predict how chromosome configurations are altered in response to changes in nuclear mechanical properties following cues from the microenvironment. In addition, it would be interesting to study how cell type-specific gene clusters are established during cellular differentiation and how they enable mechanosensitive regulation of gene expression. As most diseases are thought to arise from alterations in the local environment of the cell, it is essential to understand how chromosome and gene neighbourhoods are reorganized during disease onset to improve the development and use of precise diagnostic biomarkers and therapeutics.

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