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Cytoskeletal safeguards: Protecting the nucleus from mechanical perturbations

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Abstract

The cell nucleus plays a key role in cellular mechanoresponses. 3D genome organisation, gene expression, and cell behaviour, in general, are affected by mechanical force application to the nucleus, which is transmitted from the cellular environment via a network of interconnected cytoskeletal components. To effectively regulate cell responses, these cytoskeletal components must not only exert forces but also withstand external forces when necessary. This review delves into the latest research concerning how the cytoskeleton safeguards the nucleus from mechanical perturbations. Specifically, we focus on the three primary cytoskeletal polymers: actin, intermediate filaments, and microtubules, as well as their interactions with the cell nucleus. We discuss how the cytoskeleton acts as a protective shield for the nucleus, ensuring structural integrity and conveying context-specific mechanoresponses.

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Introduction

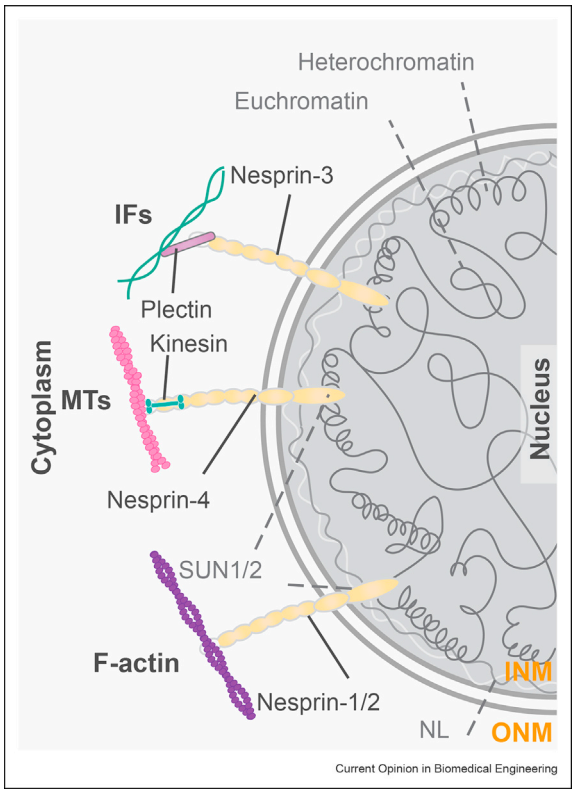
Cellular processes are modulated by biochemical and mechanical signals, which are transduced through intricate networks of molecular interactions that convey space- and time-specific responses. For biochemical signalling, this specificity is achieved by the coordinated orchestration of exciting and inhibitory signals, ensuring the fine-tuning of cellular responses. However, how mechanical signals can achieve such fine-tuning is not well understood. For this to happen, both mechano-transmitting and mechano-protecting circuits must be established.

Different subcellular structures are involved in cell response to mechanical signals (i.e., mechano-transduction), but the nucleus is particularly relevant due to its ability to receive and respond to mechanical inputs by changes in chromatin regulation and gene expression [1]. As the largest cellular organelle, it forms several interactions with the surrounding cytoskeleton and other intracellular structures. This makes it particularly amenable to mechanical perturbations and signalling. Therefore, regulatory networks that allow and prohibit nuclear mechano-transmission are essential. Mechano-inhibitory mechanisms (also termed mechano-protective) can act either by inhibiting mechanical forces from reaching the nucleus or by altering the mechanoresponsiveness of the nucleus itself (through the nuclear lamina and chromatin). Here, we will review recent work on the role of the cell cytoskeleton in nuclear mechanoprotection. For the role of the nuclear lamina and chromatin, we refer the reader to some recent reviews on the subject [1–4].

LINC complex

Cells are constantly subjected to mechanical forces transmitted from neighbouring cells and the extracellular matrix or arising from physical confinement during cell migration. Mechanical forces are transmitted to the cell interior through the cell cytoskeleton and can reach the nucleus through cytoskeletal-nuclear interactions [5]. These interactions occur at the Linker of Nucleoskeleton and Cytoskeleton (LINC) complexes [6]. These macromolecular complexes are at the interface between the outer and inner nuclear membranes and connect the cytoskeleton to the nuclear lamina and the underlying chromatin (Figure 1). At the inner nuclear membrane (INM), the Sad1 and UNC-84 (SUN) domain-containing proteins bind to the nuclear lamina (NL) and chromatin [7]. SUN proteins bind in turn to the C-terminal KASH domain of Nesprin proteins, which cross the outer nuclear membrane (ONM). Nesprins can form interactions with multiple cytoskeletal elements through the N-terminal domains. Nesprins-1 and -2 bind to F-actin through calponin homology domains (CHDs) [8]. Similarly, Nesprin-3 binds to intermediate filaments (IFs) via plectin [9] and Nesprin-4 binds to microtubules via kinesin-1 [10]

Figure 1



Nucleocytoskeletal coupling. The cell nucleus is connected to the cytoskeleton through the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. This consists of two protein families: the **N**uclear **e**nvelope **sp**ectrin-**r**epeat (Nesprins) proteins at the outer nuclear membrane (ONM) and the **S**ad1p-**UN**C-84 (SUN) domain proteins at the inner nuclear membrane (INM). There are different Nesprin proteins interacting with cytoskeletal components such as actin filaments, intermediate filaments and microtubules. Finally, SUN1/2 proteins interact with the nuclear lamina consisting of proteins of the Lamin family and with the underlying chromatin.

(Figure 1). An intact LINC complex allows the transmission of forces from the nuclear exterior to the NL and chromatin [6,11]. Therefore, tight regulation of the LINC complex or their interaction with the different cytoskeletal components ensures context-specific force transmission to the nucleus.

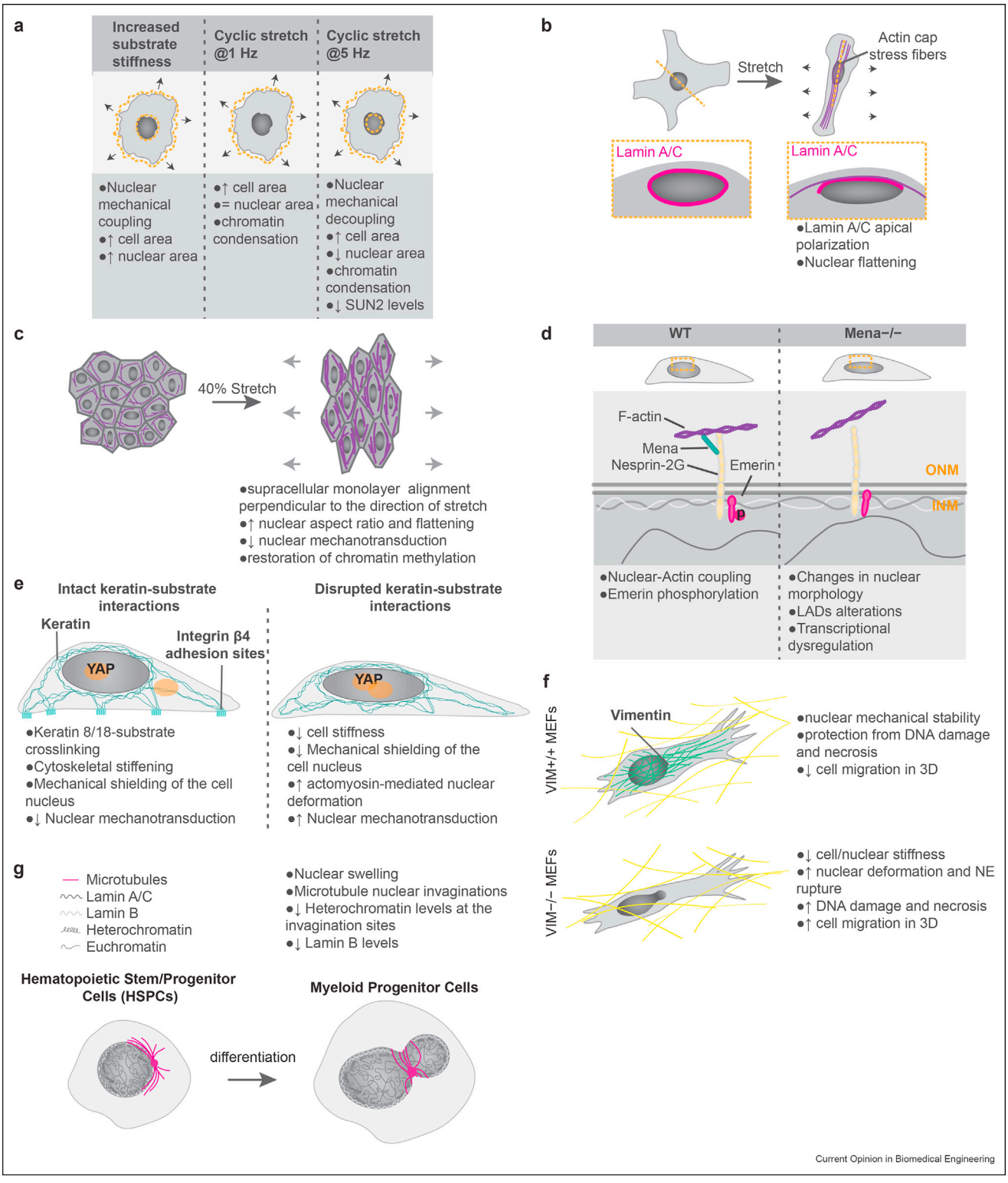
By controlling the cytoskeletal-nuclear connection and, therefore, force transmission, the LINC complex can prohibit or permit mechanical signal transduction. An active nucleocytoskeletal coupling during cell stretching will lead to nuclear strain [12]. To protect their nuclei from mechanical loading, human mesenchymal stem cells (hMSCs) were shown to respond to high-frequency (5 Hz) cell stretching by decoupling the nucleus from the cytoskeleton, leading to nuclear area reduction (as assessed from 2D images), chromatin condensation and reduced DNA damage. Downregulation of SUN2 led to lower levels of nuclear contraction and chromatin condensation [13] (Figure 2a). Interestingly, this response to high frequency differed from the cell response to lower stretch frequencies (1Hz) or substrate stiffness, indicating that both the rate and the magnitude of mechanical force regulate nuclear mechanical coupling (Figure 2a). Endothelial cells respond to cyclic strain by reorienting perpendicular to the axis of applied strain (also in a frequency-dependent way), maintaining homeostatic tensile stresses through cytoskeletal rearrangements [14]. This required the presence of Nesprin-1; in its absence, nuclei were not mechanically engaged, as indicated by the lack of nuclear flatness, and cells had increased focal adhesions and substrate traction forces. Accordingly, depletion of Nesprin-1 or Nesprin-2 increased endothelial cell and nuclear area and stimulated stress fibre assembly [15,16]. However, in a more recent study, a dominant negative Nesprin construct (DN-KASH) that displaces endogenous Nesprins-1/2 from the nuclear membrane led to reduced cell adhesion and stress fibre formation as well as defective barrier formation with altered adherens junction mechanics [17]. These findings suggest a possible redundancy in the function of different Nesprin isoforms and point to an inside-out mechanotransduction mechanism coordinated by the LINC complex.

Similarly, interference with SUN proteins leads to lower tractions and RhoA activity, indicating that they support the maintenance of a higher tension status within cells. Interestingly though, this finding seems to be cell and context-specific. SUN1/2 depletion in Vascular smooth muscle cells (VSMCs) resulted in a decreased cell area, RhoA activity and traction forces [18]. SUN2 has also been shown to promote RhoA activity in HeLa cells. However, SUN1 antagonised Sun2 LINC complexes and SUN1 depletion resulted in higher RhoA activity and vinculin recruitment at focal adhesions [19]. In another study, although integrin recruitment at the cell surface of HeLa cells was unaffected, SUN1 depletion reduced focal adhesion maturation [20]. This discrepancy could be attributed to sample preparation. In the case of higher vinculin recruitment at the focal adhesions [19], cells were cultured on fibronectin-coated slides, raising the possibility of integrin-specific responses related to SUN1 activity. Engagement of different integrins can, in turn, propagate forces to the nucleus via diverse "routes" involving different cytoskeletal elements.

Actomyosin cytoskeleton

As a dynamic and structural element of cells, the actomyosin cytoskeleton regulates nuclear mechanoresponses by promoting nuclear deformation and supporting nuclear structural integrity [21]. Forces arising from the cell-ECM interface propagate through actin to the nucleus, causing conformational changes to chromatin and transcriptional regulation [22].

Figure 2



Examples of nuclear mechanoprotection from the cell cytoskeleton. a. Cells respond to high-frequency cyclic stretch (5Hz) by decoupling of the cell nucleus, mediated by a reduction in SUN2 levels. The responses are different to those mediated in response to stiffness or to lower frequency stretching (1Hz), where the nucleus is mechanically coupled or does not respond to cell strain changes, respectively [13]. **b**. Lamin A/C expressing MEFs form an actin cap to resist nuclear deformation, preserving nuclear integrity from mechanical stretching [29]. **c**. Supracellular monolayer alignment prevents nuclear mechanotransduction, facilitating chromatin restoration and minimising the stress-induced load on adhesion structures, resulting in mechanoprotection of EPC monolayers [30]. **d**. Mena in controlling nuclear architecture, chromatin repositioning, and gene expression via

Therefore, several mechanisms need to be in place to regulate how and to which extent mechanical forces from the actin cytoskeleton reach the nucleus. In non-muscle cells, contractile forces are predominantly generated by the action of myosin II motors on actin filaments. Unrestrained contractility caused by loss of the protein phosphatase 1 (PP1) complex subunit PPP1R12A elicits nuclear envelope rupture and drives genome instability [23]. The dynamic nature of actin regulation also helps cells to adapt to mechanical signals of different magnitudes, conveying context-specific responses. Increasing loading rates trigger nuclear mechanoresponses, as indicated by nuclear YAP localisation. However, the actin cytoskeleton softens above a given threshold, decreasing loading rates and preventing mechanotransduction [24]. Likewise, mouse embryonic skin fibroblasts (MSFs) showed opposed responses to different strain levels. Nuclear areas were increased, and chromatin compaction decreased for low strains (5%), while the opposite was observed for high strains (20%) [25]. The actin cytoskeleton was essential for both these responses, and its disruption increased the occurrence of double-strand breaks during stretch. These findings suggest that mechanical confinement of nuclei by actin may be protective during high mechanical stretch or loading.

Cells exposed to high mechanical forces, such as fibroblasts and muscle cells, often possess a dome-shaped actin cap covering the top of the nucleus. This actin cap is made up of contractile actin filament bundles containing phosphorylated myosin, which creates a highly organised and dynamic structure [21]. Actin fibres forming the actin cap are connected to the ECM through focal adhesions and to the nucleus via the LINC complex [26,27]. Lamin A/C is required to form the perinuclear actin cap, as cells expressing LMNA mutations or lacking the LMNA gene do not form an actin cap [21].

Cells respond to uniaxial cyclic stretch by reorienting their cytoskeleton to dissipate forces by rebuilding and remodelling relevant internal structural components such as the actin cap [28]. Upon mechanical loading, the actin cap transmits mechanical stress to the lamin A/C-bound nucleus causing an apically polarised lamin A/C. Inhibiting myosin contractility or depleting Nesprin-2G inhibits the formation of thick actin cap fibres, leading to altered nuclear morphology. Using computer simulations, Kim et al. demonstrated that upon cell stretch, the actin cap absorbs part of the applied mechanical stress, directing it away from the nucleus [29](Figure 2b). Similar redistribution of intracellular forces by supracellular actin rearrangements is observed in epidermal progenitor cells (EPCs). These cells respond to uniaxial stretch by reorienting F-actin and nuclei perpendicular to the direction of the stretch to prevent force transmission to the nucleus and nuclear mechanotransduction [30] (Figure 2c).

Some cells generate a perinuclear actin rim in response to mechanical perturbations. Local application of force can prompt a transient Ca^{2+}-dependent accumulation of actin in the perinuclear area [31]. Failure to elicit this response results in aberrant mechanoresponses and DNA damage [30] in EPCs. Moreover, strain-induced accumulation of perinuclear actin can help maintain EPCs in an undifferentiated state by regulating the availability of nuclear G-actin and altering chromatin compaction [32]. Indeed, the rate of actin polymerisation at the cytoplasm is an effective way to regulate G-actin pools within the nucleus, thus regulating gene expression and chromatin organisation (reviewed in [33,34]). More recently, the interaction of Nesprin-2G with actin via the protein Mena was shown to affect nuclear architecture in both 2D and 3D environments. Depletion of Mena leads to dysregulation of genes associated with cell adhesion, migration, ECM organisation, and immune response, partly due to altered chromatin organisation resulting from changes in emerin phosphorylation and lamina–chromatin interactions [35](Figure 2b).

Both G- and F-actin have been shown to convey protection from DNA damage and regulate chromatin organisation within the nucleus [33]. However, the contribution of nuclear actin in nuclear mechanoprotection is still poorly understood. Some evidence stems from recent studies illustrating that nuclear F-actin counteracts nuclear envelope deformation during replication stress and promotes repair [36]. Similarly, during mitotic exit, a dynamic and transient F-actin assembly promotes nuclear volume expansion and chromatin decondensation [37].

Cytoplasmic IFs

Intermediate filaments (IFs) are an essential component of the cell cytoskeleton and the nucleus contributing to their stiffness and organization [3,38,39]. IFs display tissue- and context-specific expression, and changes in their protein composition or levels often indicate cell fate transitions [40]. IFs span from cell-ECM and cell–cell contacts to the nucleus, where they directly bind via plectin-Nesprin-3 interactions [9] (Figure 1). They also form dynamic interactions with actin filaments and microtubules. Despite being the softest component of

direct signalling across the nuclear envelope through emerin phosphorylation [35]. **e**. Keratin-substrate interactions increase cytoskeletal stiffness preventing actomyosin-mediated nuclear deformation and reducing YAP nuclear translocation [47]. **f**. Perinuclear Vimentin increases nuclear stiffness and protects nuclei from mechanical rupture and DNA damage during confined cell migration [55,56]. **g**. Microtubules constrain the swelling nuclei of myeloid progenitor cells during their differentiation from Hematopoietic Stem/Progenitor Cells (HSPCs), locally affecting nuclear lamina composition and chromatin organisation [61].

the cytoskeleton, IFs can withstand greater mechanical deformation than actin and microtubules, with their elastic modulus increasing at large strains [38,41]. The rheological properties of IFs make them essential force-bearing elements of the cytoskeleton, capable of withstanding forces and adjusting their mechanical properties in response to mechanical signalling. This can affect the distribution and maintenance of forces within cells, ultimately affecting nuclear mechanoresponses. Interestingly, IF mechanical properties can be tuned through different mechanisms. First, variations in the amino acid sequence of different types of IFs result in diverse patterns of charge and hydrophobicity, which can, in turn, affect their mechanics [42]. Second, IFs can crosslink and form bundles and other structures of increased rigidity in the presence of physiological concentrations of divalent cations such as $Mg2+$ or $Ca2+$ [43]. This suggests that IF mechanics can be highly regulated, potentially affecting nuclear mechanoprotection.

Keratin IFs

Matrix stiffness impacts the mechanical properties of the keratin network in keratinocytes [44]. Higher substrate rigidity resulted in a more interconnected keratin network with higher stiffness. Destabilization of Keratin-14 through the R416P mutation (responsible for Dowling-Meara form of epidermolysis bullosa simplex (EBS)) caused structural remodelling of the keratin cytoskeleton and decreased cell stiffness. This, in turn, led to changes to nuclear mechanoresponses, indicated by the lower levels of nuclear LaminA/C levels on stiffer substrates [44]. This effect of stiffness on lamin is opposite to that typically reported [45], a discrepancy possibly due to perturbations on the organisation of the actin cytoskeleton. Indeed, in keratinocytes lacking plectin (the primary crosslinker of keratin to other cellular components and the nucleus), increased lamin A/C was associated with increased formation of F-actin stress fibres [44]. Disrupting plectin, or loss of the entire type I keratin locus, also leads to increased nuclear mechanoresponses [44,46]. Subsequently, in individuals bearing plectin mutations causing EBS with muscular dystrophy (EBS-MD), nuclei become more responsive to actomyosin-mediated nuclear deformation with characteristic abnormal nuclear morphology in skin biopsies [46]. Furthermore, the interaction between the keratin network and the cellular environment can affect the organisation and mechanics of the keratin cytoskeleton. We have recently shown that crosslinking keratin to a laminin extracellular matrix through hemidesmosomes increases cytoskeletal stiffness, shielding the nucleus from actomyosin-mediated mechanical deformation [47] (Figure 2e). This nuclear mechanoprotection is further observed *in vivo*, where Keratin-8 levels are inversely correlated with nuclear deformation in invasive breast ductal carcinoma biopsies. Relatedly, Keratin-8 loss in mouse colonocytes changed nuclear morphology *in vivo* and compromised nuclear integrity in colorectal adenocarcinoma cells. Shear stress further exacerbated this phenotype [48].

Vimentin IFs

Vimentin is one of the most studied IF, present in many different cell types, providing mechanical support and maintaining cell shape. Vimentin, similar to keratin networks, forms direct and dynamic interactions with other cytoskeletal elements, which can impact force generation, actin retrograde flows, and cell migration [49,50]. The effects of these interactions depend on the mechanical and chemical composition of the cellular environment, with vimentin potentially increasing or decreasing cell-generated forces [51,52]. Vimentin helps to homogenise intracellular stress distribution and slows actin stress fibre retrograde flows [49]. This is consistent with its role as a mechanoprotective network that absorbs and redistributes forces within cells to protect against actin-mediated nuclear deformation. Accordingly, the interpenetrating network of the vimentin-actin cytoskeleton increases contractile forces and contributes to cell resilience upon stretching [53]. Interestingly, vimentin expression levels are affected differently by the cell stretch frequency. While low frequencies (1Hz) increase the levels of vimentin expression, high-frequency stretch (5 Hz) led to its downregulation, concomitantly with a decrease in nuclear mechanical coupling [13].

Due to their mechanical properties and surface charges, vimentin intermediate filament networks stiffen in both compression and extension. Mouse embryonic fibroblasts (MEFs) demonstrate compression stiffening and can resist repeated compression cycles, protecting their nuclei from damage. In contrast, vimentin null fibroblasts have more nuclei with blebs or visible nuclear membrane disruption under compressive loads [54]. Patteson et al. demonstrated that the vimentin network confers mechanical stability to the nucleus, preventing nuclear rupture and protecting against DNA damage during confined cell migration [55,56](Figure 2f).

Desmin IFs

Desmin IFs are primarily found in muscle cells, where they maintain their structural integrity by forming a network of filaments that connect the contractile machinery of the cell to the structural elements of the cell membrane. Desmin filaments can further connect to the nucleus via Nesprin-3-plectin interactions. This interaction is essential for maintaining the typical spheroidal architecture of muscle cell nuclei and their proper positioning and movement along the myofiber [57]. Further depletion of desmin results in severe lamina wrinkling and infolding, affecting chromatin organisation [58].

Microtubules

Microtubules are the most rigid of cytoskeletal polymers. They can sustain compressive forces to a certain extent, maintaining the cell's shape and mechanical resistance [59]. Microtubules can form a cage-like structure surrounding the nucleus during interphase, impacting nuclear morphology and overall genome integrity [60]. This structure often produces invaginations of the nucleus, dictating nuclear and chromatin organisation. For instance, microtubule-dependent forces induce characteristic invaginations by constraining the swelling nucleus of human Hematopoietic Stem and Progenitor Cells (HSPCs) during early commitment toward the myeloid lineage. This affects chromatin organisation and nuclear lamina density [61], allowing eventually altered gene expression (Figure 2g). Similarly, during drosophila cellularisation, microtubules are concentrated at nuclear grooves. They are essential for nuclear envelope remodelling from spherical to elongated and lobulated morphologies [62], influencing the underlying chromatin organisation. This perinuclear microtubule cage also accumulates around the nucleus in areas of cellular constriction in a process mediated by Cytoplasmic linker-associated proteins (CLASPs). The absence of CLASPs prevents microtubules from resisting mechanical compression, leading to depolymerisation, cell rupture, and ultimately, cell death [63].

The interactions of microtubules with other cytoskeletal elements also contribute to maintaining cellular integrity and reducing force transmission to the nucleus. Microtubules can buckle under actomyosin contractile stresses and are able to bear compressive loads in living cells [64]. The depolymerisation of microtubules increases cell contractility, which reduces nuclear height and subsequently stretches and stiffens the nuclear lamina network [65–67]. The impact of anti-cytoskeletal drugs revealed that actin filaments and microtubules have both redundant and unique functions in stiffening the nucleus and slowing strain recovery. Interfering with both actin filaments and microtubules resulted in softer nuclei than those in which only one was disrupted, highlighting their individual roles in preventing extreme nuclear deformations. Nevertheless, the viscosity of nuclei treated with both drugs was similar to that of nuclei treated with either drug alone, emphasising the need for both actin filaments and microtubules to maintain high viscosity and slow down the strain recovery process [68].

Maintaining nuclear shape and lamina integrity requires a balance between microtubules and intermediate filaments [69,70]. In cardiomyocytes, desmin maintains nuclear size while microtubules compress the nucleus along its long axis, and disruption of either component causes changes in nuclear morphology [52]. Interfering with desmin or Nesprin-3 leads to collapsed nuclei, which is prevented by microtubule depolymerisation [58]. Balanced interactions between the cytoskeletal and lamin networks are also necessary for normal nuclear shape. In MEFs, the absence of Lamin A,C leads to irregular nuclear shape mediated by the microtubule network [70]. Accordingly, the microtubule organising centre (MTOC) can exert pushing forces on the nucleus and cause nuclear deformations under low tension/stiffness conditions. The increase of nuclear stiffness by lamin A,C overexpression partially reverses this phenotype [65]. MTOC-nuclear interactions can further trigger heterochromatin accumulation. This increase in heterochromatin can lead to a local increase in nuclear stiffness, possibly protecting both the nuclear envelope and the underlining chromatin from physical damage [62,71,72].

Conclusions and outlook

The nucleus and the genome must be selectively and specifically responsive to extracellular mechanical signals. This is accomplished by the precise regulation of intracellular force transmission. The cell cytoskeleton forms a specialised circuit which conveys cell and context-specific mechanoresponses to the nucleus, orchestrating force transmission or mechanoprotection. While actin and microtubules play a more dynamic role in transducing and applying forces to the nuclei, the cell intermediate filaments network integrates mechanical forces to alter cytoskeletal mechanics. However, it's important to consider the different cytoskeletal elements as parts of an interactive polymer whose mechanical properties and mechanoresponsiveness differ from those of its constituent networks.

The specific combination of cytoskeletal components, their interaction with the extracellular environment, and the nucleus and nuclear mechanical properties ultimately determine nuclear mechanoresponses. This gives each cell type a unique mechanical barcode that offers context-specific mechanoresponses that can either transduce or hinder mechanical signals, thereby regulating downstream responses.

CRediT author statement

Zanetta Kechagia: Writing- Original draft preparation. Writing- Reviewing and Editing. Pere Roca-Cusachs: Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

1. Kalukula Y, Stephens AD, Lammerding J, Gabriele S: **Mechanics and functional consequences of nuclear deformations**. *Nat Rev Mol Cell Biol* 2022, **23**:583–602.

2. Wang M, Ivanovska I, Vashisth M, Discher DE: **Nuclear mechanoprotection: from tissue atlases as blueprints to distinctive regulation of nuclear lamins**. *APL Bioeng* 2022, **6**, 21504.

3. Tenga R, Medalia O: **Structure and unique mechanical aspects of nuclear lamin filaments**. *Curr Opin Struct Biol* 2020, **64**: 152–159.

4. Stephens AD, Banigan EJ, Marko JF: **Chromatin's physical properties shape the nucleus and its functions**. *Curr Opin Cell Biol* 2019, **58**:76–84.

5. Kechagia JZ, Ivaska J, Roca-Cusachs P: **Integrins as biomechanical sensors of the microenvironment**. *Nat Rev Mol Cell Biol* 2019, **20**.

6. Lombardi ML, Jaalouk DE, Shanahan CM, Burke B, Roux KJ, Lammerding J: **The interaction between nesprins and Sun proteins at the nuclear envelope is critical for force transmission between the nucleus and cytoskeleton**. *J Biol Chem* 2011, **286**, 26743.

7. Haque F, Lloyd DJ, Smallwood DT, Dent CL, Shanahan CM, Fry AM, Trembath RC, Shackleton S: **SUN1 interacts with nuclear lamin A and cytoplasmic nesprins to provide a physical connection between the nuclear lamina and the cytoskeleton**. *Mol Cell Biol* 2006, **26**:3738–3751.

8. Crisp M, Liu Q, Roux K, Rattner JBB, Shanahan C, Burke B, Stahl PD, Hodzic D. *Coupling of the nucleus and cytoplasm: role of the LINC complex*, **172**; 2006:41–53.

9. Wilhelmsen K, Litjens SHM, Kuikman I, Tshimbalanga N, Janssen H, Van Bout I Den, Raymond K, Sonnenberg A: **Nesprin-3, a novel outer nuclear membrane protein, associates with the cytoskeletal linker protein plectin**. *J Cell Biol* 2005, **171**: 799–810.

10. Roux KJ, Crisp ML, Liu Q, Kim D, Kozlov S, Stewart CL, Burke B: **Nesprin 4 is an outer nuclear membrane protein that can induce kinesin-mediated cell polarization**. *Proc Natl Acad Sci U S A* 2009, **106**:2194–2199.

11. Guilluy C, Osborne LD, Van Landeghem L, Sharek L, Superfine R, Garcia-Mata R, Burridge K: **Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus**. *Nat Cell Biol* 2014, **16**:376–381.

12. Elosegui-Artola A, Andreu I, Beedle AEM, Lezamiz A, Uroz M, Kosmalska AJ, Oria R, Kechagia JZ, Rico-Lastres P, Le Roux A-LA-L, *et al.*: **Force triggers YAP nuclear entry by regulating transport across nuclear pores**. *Cell* 2017, **171**: 1397–1410.e14.

13. Gilbert HTJ, Mallikarjun V, Dobre O, Jackson MR, Pedley R, Gilmore AP, Richardson SM, Swift J: **Nuclear decoupling is part of a rapid protein-level cellular response to high-intensity mechanical loading**. *Nat Commun* 2019, **10**:1–15.

14. Stamenović D, Krishnan R, Canović EP, Smith ML: **As the endothelial cell reorients, its tensile forces stabilize**. *J Biomech* 2020, **105**, 109770.

15. King SJ, Nowak K, Suryavanshi N, Holt I, Shanahan CM, Ridley AJ: **Nesprin-1 and nesprin-2 regulate endothelial cell shape and migration**. *Cytoskeleton* 2014, **71**:423–434.

16. Chancellor TJ, Lee J, Thodeti CK, Lele T, Chancellory TJ, Lee J, Thodeti CK, Lele T: **Actomyosin tension exerted on the nucleus through nesprin-1 connections influences endothelial cell adhesion, migration, and cyclic strain-induced reorientation**. *Biophys J* 2010, **99**:115–123.

17. Denis KB, Cabe JI, Danielsson BE, Tieu KV, Mayer CR, Conway DE: **The LINC complex is required for endothelial cell adhesion and adaptation to shear stress and cyclic stretch**. *Mol Biol Cell* 2021, **32**:1654–1663.

18. Porter L, Minaisah RM, Ahmed S, Ali S, Norton R, Zhang Q, Ferraro E, Molenaar C, Holt M, Cox S, *et al.*: **SUN1/2 are essential for RhoA/ROCK-regulated actomyosin activity in isolated vascular smooth muscle cells**. *Cells* 2020, **9**.

19. Thakar K, May CK, Rogers A, Carroll CW: **Opposing roles for distinct LINC complexes in regulation of the small GTPase RhoA**. *Mol Biol Cell* 2017, **28**:182–191.

20. Ueda N, Maekawa M, Matsui TS, Deguchi S, Takata T, Katahira J, Higashiyama S, Hieda M: **Inner nuclear membrane protein, SUN1, is required for cytoskeletal force generation and focal adhesion maturation**. *Front Cell Dev Biol* 2022:10.

21. Khatau SB, Hale CM, Stewart-Hutchinson PJ, Patel MS, Stewart CL, Searson PC, Hodzic D, Wirtz D: **A perinuclear actin cap regulates nuclear shape**. *Proc Natl Acad Sci U S A* 2009, **106**: 19017–19022.

22. Tajik A, Zhang Y, Wei F, Sun J, Jia Q, Zhou W, Singh R, Khanna N, Belmont AS, Wang N: **Transcription upregulation via force-induced direct stretching of chromatin**. *Nat Mater* 2016, **15**:1287–1296.

23. Takaki T, Montagner M, Serres MP, Le Berre M, Russell M, Collinson L, Szuhai K, Howell M, Boulton SJ, Sahai E, *et al.*: **Actomyosin drives cancer cell nuclear dysmorphia and threatens genome stability**. *Nat Commun* 2017, **8**.

24. Andreu I, Falcones B, Hurst S, Chahare N, Quiroga X, Le Roux A-L, Kechagia Z, Beedle AEM, Elosegui-Artola A, Trepat X, *et al.*: **The force loading rate drives cell mechanosensing through both reinforcement and cytoskeletal softening**. *Nat Commun* 2021, **12**:4229.

25. Seelbinder B, Scott AK, Nelson I, Schneider SE, Calahan K, Neu CP: **TENSCell: imaging of stretch-activated cells reveals divergent nuclear behavior and tension**. *Biophys J* 2020, **118**: 2627–2640.

26. Sawada Y, Tamada M, Dubin-Thaler BJBJ, Cherniavskaya O, Sakai R, Tanaka S, Sheetz MPMP, Balaban NQ, Schwarz US, Riveline D, *et al.*: **No title**. *Cell* 2006, **127**.

27. Woroniuk A, Porter A, White G, Newman DT, Diamantopoulou Z, Waring T, Rooney C, Strathdee D, Marston DJ, Hahn KM, *et al.*: **STEF/TIAM2-mediated Rac1 activity at the nuclear envelope regulates the perinuclear actin cap**. *Nat Commun* 2018, **9**: 1–15.

28. Livne A, Bouchbinder E, Geiger B: **Cell reorientation under cyclic stretching**. *Nat Commun* 2014, **5**:1–8.

29. Kim JK, Louhghalam A, Lee G, Schafer BW, Wirtz D, Kim DH: **Nuclear lamin A/C harnesses the perinuclear apical actin cables to protect nuclear morphology**. *Nat Commun* 2017, **8**: 1–13.

30. Nava MM, Miroshnikova YA, Biggs LC, Whitefield DB, Metge F, Boucas J, Vihinen H, Jokitalo E, Li X, García Arcos JM, *et al.*: **Heterochromatin-driven nuclear softening protects the genome against mechanical stress-induced damage**. *Cell* 2020, **181**:800–817.e22.

31. Shao X, Li Q, Mogilner A, Bershadsky AD, Shivashankar GV: **Mechanical stimulation induces formin-dependent assembly**

of a perinuclear actin rim. *Proc Natl Acad Sci U S A* 2015, **112**: E2595–E2601.

32. Le HQ, Ghatak S, Yeung CYC, Tellkamp F, Günschmann C, Dieterich C, Yeroslaviz A, Habermann B, Pombo A, Niessen CM, *et al.*: **Mechanical regulation of transcription controls Polycomb-mediated gene silencing during lineage commitment**. *Nat Cell Biol* 2016, **18**:864–875.

33. Ulferts S, Prajapati B, Grosse R, Vartiainen MK: **Emerging properties and functions of actin and actin filaments inside the nucleus**. *Cold Spring Harbor Perspect Biol* 2021, **13**:1–16.

34. Green NM, Kimble GC, Talbot DE, Tootle TL: **Nuclear actin**. In *eLS*. Wiley; 2021:958–967.

35. * Li Mow Chee F, Beernaert B, Griffith BGC, Loftus AEP, Kumar Y, Wills JC, Lee M, Valli J, Wheeler AP, Armstrong JD, *et al.*: **Mena regulates nesprin-2 to control actin–nuclear lamina associations, trans-nuclear membrane signalling and gene expression**. *Nat Commun* 2023, **14**:1602.
This study identifies Mena, an actin regulatory protein, as an integrin adhesion complex element that can interact with nesprin-2 at the nuclear envelope to modulate nuclear architecture, chromatin repositioning, and gene expression. This highlights a novel role for adhesion proteins in direct signalling across the nuclear envelope and provides an example of how perinuclear actin can regulate gene expression.

36. Lamm N, Read MN, Nobis M, Van Ly D, Page SG, Masamsetti VP, Timpson P, Biro M, Cesare AJ: **Nuclear F-actin counteracts nuclear deformation and promotes fork repair during replication stress**. *Nat Cell Biol* 2020, **22**: 1460–1470.

37. Baarlink C, Plessner M, Sherrard A, Morita K, Misu S, Virant D, Kleinschnitz EM, Harniman R, Alibhai D, Baumeister S, *et al.*: **A transient pool of nuclear F-actin at mitotic exit controls chromatin organization**. *Nat Cell Biol* 2017, **19**:1389–1399.

38. Charrier EE, Janmey PA: **Mechanical properties of intermediate filament proteins**. In *Methods in enzymology*. Academic Press Inc.; 2016:35–57.

39. Bordeleau F, Lapierre ME, Sheng Y, Marceau N: **Keratin 8/18 regulation of cell stiffness-extracellular matrix interplay through modulation of rho-mediated actin cytoskeleton dynamics**. *PLoS One* 2012, **7**, 38780.

40. Redmond CJ, Coulombe PA: *Intermediate filaments as effectors of differentiation*. 2021.

41. * Elbalasy I, Mollenkopf P, Tutmarc C, Herrmann H, Schnauß J: **Keratins determine network stress responsiveness in reconstituted actin–keratin filament systems**. *Soft Matter* 2021. https://doi.org/10.1039/d0sm02261f.
Using *in vitro* reconstituted actin and keratin 8/18 composite filament networks, this study found that for relatively small deformations, composite networks exhibited properties that were intermediate between actin and keratin. For larger deformations, keratin addition to actin networks increased strain stiffening behaviour, implying a stiffening role of keratin.

42. Schepers AV, Lorenz C, Köster S: **Tuning intermediate filament mechanics by variation of pH and ion charges**. *Nanoscale* 2020, **12**:15236–15245.

43. Lin YC, Broedersz CP, Rowat AC, Wedig T, Herrmann H, MacKintosh FC, Weitz DA: **Divalent cations crosslink vimentin intermediate filament tail domains to regulate network mechanics**. *J Mol Biol* 2010, **399**:637–644.

44. * Laly AC, Sliogeryte K, Pundel OJ, Ross R, Keeling MC, Avisetti D, Waseem A, Gavara N, Connelly JT: **The keratin network of intermediate filaments regulates keratinocyte rigidity sensing and nuclear mechanotransduction**. *Sci Adv* 2021, **7**: 6187–6214.
This study demonstrated that keratinocytes respond to increasing matrix stiffness by forming a rigid network of keratin bundles and actin stress fibres. Disrupting keratin stability led to reduced stress fibres and cell stiffness and impaired mechanotransduction to the nuclear lamina, which mediates stiffness-dependent chromatin remodelling.

45. Swift J, Ivanovska IL, Buxboim A, Harada T, Dingal PCDP, Pinter J, Pajerowski JD, Spinler KR, Shin JW, Tewari M, *et al.*: **Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation**. *Science* 2013:341 (80-).

46. Almeida FV, Walko G, McMillan JR, McGrath JA, Wiche G, Barber AH, Connelly JT: **The cytolinker plectin regulates nuclear mechanotransduction in keratinocytes**. *J Cell Sci* 2015, **128**:4475–4486.

47. * Kechagia Z, Sáez P, Gómez-González M, Zamarbide M, Andreu I, Koorman T, Beedle AEM, Derksen PWB, Trepat X, Arroyo M, *et al.*: **The laminin-keratin link shields the nucleus from mechanical deformation and signalling**. *Nat Mater* 2023. https://doi.org/10.1038/s41563-023-01657-3.
This study identified a mechanism by which cells shield nuclear mechanoresponses in response to matrix stiffness when cultured on a laminin substrate. The mechanism involves the cross-linking of keratin to laminin through β4 integrins, stiffening the cytoskeleton and preventing actin-mediated nuclear deformation.

48. * Stenvall C-GA, Nyström JH, Butler-Hallissey C, Jansson T, Heikkilä TRH, Adam SA, Foisner R, Goldman RD, Ridge KM, Toivola DM: **Cytoplasmic keratins couple with and maintain nuclear envelope integrity in colonic epithelial cells**. *Mol Biol Cell* 2022, **33**.
This study demonstrates, both in cells and *in vivo*, that Keratin 8 plays a crucial role in maintaining the composition of the nuclear envelope and lamina in colonocytes, contributing to mechanical stability, protection against stress, and the nuclear integrity of epithelial cells.

49. Costigliola N, Ding L, Burckhardt CJ, Han SJ, Gutierrez E, Mota A, Groisman A, Mitchison TJ, Danuser G: **Vimentin fibers orient traction stress**. *Proc Natl Acad Sci U S A* 2017, **114**:5195–5200.

50. Pora A, Yoon S, Dreissen G, Hoffmann B, Merkel R, Windoffer R, Leube RE: **Regulation of keratin network dynamics by the mechanical properties of the environment in migrating cells**. *Sci Rep* 2020, **10**:1–17.

51. Alisafaei F, Mandal K, Swoger M, Yang H, Guo M, Janmey PA, Patteson AE, Shenoy VB: **Vimentin intermediate filaments can enhance or abate active cellular forces in a microenvironmental stiffness-dependent manner**. *bioRxiv* 2022. https://doi.org/10.1101/2022.04.02.486829.

52. Van Loosdregt IAEW, Weissenberger G, Van Maris MPFHL, Oomens CWJ, Loerakker S, Stassen OMJA, Bouten CVC: **The mechanical contribution of vimentin to cellular stress generation**. *J Biomech Eng* 2018:140.

53. Wu H, Shen Y, Sivagurunathan S, Weber MS, Adam SA, Shin JH, Fredberg JJ, Medalia O, Goldman R, Weitz DA: **Vimentin intermediate filaments and filamentous actin form unexpected interpenetrating networks that redefine the cell cortex**. *Proc Natl Acad Sci U S A* 2022, **119**, e2115217119.

54. Pogoda K, Byfield F, Deptuła P, Cieśluk M, Suprewicz Ł, Skłodowski K, Shivers JL, Van Oosten A, Cruz K, Tarasovetc E, *et al.*: **Unique role of vimentin networks in compression stiffening of cells and protection of nuclei from compressive stress**. *Nano Lett* 2022, **22**:4725–4732.

55. Patteson AE, Vahabikashi A, Pogoda K, Adam SA, Mandal K, Kittisopikul M, Sivagurunathan S, Goldman A, Goldman RD, Janmey PA: **Vimentin protects cells against nuclear rupture and DNA damage during migration**. *J Cell Biol* 2019, **218**: 4079–4092.

56. Patteson AE, Pogoda K, Byfield FJ, Mandal K, Ostrowska-Podhorodecka Z, Charrier EE, Galie PA, Deptuła P, Bucki R, McCulloch CA, *et al.*: **Loss of vimentin enhances cell motility through small confining spaces**. *Small* 2019, **15**, 1903180.

57. Staszewska I, Fischer I, Wiche G: **Plectin isoform 1-dependent nuclear docking of desmin networks affects myonuclear architecture and expression of mechanotransducers**. *Hum Mol Genet* 2015, **24**:7373–7389.

58. Heffler J, Shah PP, Robison P, Phyo S, Veliz K, Uchida K, Bogush A, Rhoades J, Jain R, Prosser BL: **A balance between intermediate filaments and microtubules maintains nuclear architecture in the cardiomyocyte**. *Circ Res* 2020. https://doi.org/10.1161/CIRCRESAHA.119.315582.

59. Hawkins T, Mirigian M, Selcuk Yasar M, Ross JL: **Mechanics of microtubules**. *J Biomech* 2010, **43**:23–30.

60. Shokrollahi M, Mekhail K: **Interphase microtubules in nuclear organization and genome maintenance**. *Trends Cell Biol* 2021, **31**:721–731.

61. Biedzinski S, Agsu G, Vianay B, Delord M, Blanchoin L, Larghero J, Faivre L, Théry M, Brunet S: **Microtubules control nuclear shape and gene expression during early stages of hematopoietic differentiation**. *EMBO J* 2020, **39**, e103957.

62. Hampoelz B, Azou-Gros Y, Fabre R, Markova O, Puech PH, Lecuit T: **Microtubule-induced nuclear envelope fluctuations control chromatin dynamics in Drosophila embryos**. *Development* 2011, **138**:3377–3386.

63. Ju RJ, Falconer AD, Dean KM, Fiolka RP, Sester DP, Nobis M, Timpson P, Lomakin AJ, Danuser G, White MD, *et al.*: **Compression-dependent microtubule reinforcement comprises a mechanostat which enables cells to navigate confined environments**. *bioRxiv* 2023. https://doi.org/10.1101/2022.02.08.479516.

64. Brangwynne CP, MacKintosh FC, Kumar S, Geisse NA, Talbot J, Mahadevan L, Parker KK, Ingber DE, Weitz DA: **Microtubules can bear enhanced compressive loads in living cells because of lateral reinforcement**. *J Cell Biol* 2006, **173**:733–741.

65. Alisafaei F, Jokhun DS, Shivashankar GV, Shenoy VB: **Regulation of nuclear architecture, mechanics, and nucleocytoplasmic shuttling of epigenetic factors by cell geometric constraints**. *Proc Natl Acad Sci U S A* 2019, **116**:13200–13209.

66. Rafiq NBM, Nishimura Y, Plotnikov SV, Thiagarajan V, Zhang Z, Shi S, Natarajan M, Viasnoff V, Kanchanawong P, Jones GE, *et al.*: **A mechano-signalling network linking microtubules, myosin IIA filaments and integrin-based adhesions**. *Nat Mater* 2019, **18**:638–649.

67. Ramdas NM, Shivashankar GV: **Cytoskeletal control of nuclear morphology and chromatin o1rganization**. *J Mol Biol* 2015, **427**:695–706.

68. Wang X, Liu H, Zhu M, Cao C, Xu Z, Tsatskis Y, Lau K, Kuok C, Filleter T, McNeill H, *et al.*: **Mechanical stability of the cell nucleus - roles played by the cytoskeleton in nuclear deformation and strain recovery**. *J Cell Sci* 2018:131.

69. Borin D, Peña B, Chen SN, Long CS, Taylor MRG, Mestroni L, Sbaizero O: **Altered microtubule structure, hemichannel localization and beating activity in cardiomyocytes expressing pathologic nuclear lamin A/C**. *Heliyon* 2020, **6**, e03175.

70. Tariq Z, Zhang H, Chia-Liu A, Shen Y, Gete Y, Xiong ZM, Tocheny C, Campanello L, Wu D, Losert W, *et al.*: **Lamin A and microtubules collaborate to maintain nuclear morphology**. *Nucleus* 2017, **8**:433–446.

71. Maizels Y, Gerlitz G: **Shaping of interphase chromosomes by the microtubule network**. *FEBS J* 2015, **282**:3500–3524.

72. Furusawa T, Rochman M, Taher L, Dimitriadis EK, Nagashima K, Anderson S, Bustin M. *Chromatin decompaction by the nucleosomal binding protein HMGN5 impairs nuclear sturdiness*, **6**; 2015.