The Nuclear Envelope: LINCing tissue mechanics to genome regulation in cardiac and skeletal muscle

Authors: Rachel Piccus[1] and Daniel Brayson[2,3]

Addresses: [1]Centre for Human and Applied Physiological Sciences, King's College London, UK

[2]School of Cardiovascular Medicine and Sciences, King's College London, UK

[3]Molecular Neurosciences, UCL Great Ormond Street Institute of Child Health, London, UK

Address for correspondence: Daniel Brayson,
Molecular Neurosciences,
UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH,
United Kingdom.

Phone: +447783208383

Email: d.brayson@ucl.ac.uk.

Abstract

Regulation of the genome is viewed through the prism of gene expression, DNA replication and DNA repair as controlled through transcription, chromatin compartmentalisation and recruitment of repair factors by enzymes such as DNA polymerases, ligases, acetylases, methylases and cyclin dependent kinases. However, recent advances in the field of muscle cell physiology have also shown a compelling role for 'outside-in' biophysical control of genomic material through mechano-transduction. The crucial hub that transduces these biophysical signals is called the Linker of Nucleoskeleton and Cytoskeleton (LINC). This complex is embedded across the nuclear envelope, which separates the nucleus from the cytoplasm. How the LINC complex operates to mechanically regulate the many functions of DNA is becoming increasingly clear, and recent advances have provided exciting insight into how this occurs in cells from mechanically activated tissues such as skeletal and cardiac muscle. Nevertheless, there are still some notable shortcomings in our understanding of these processes and resolving these will likely help us understand how muscle diseases manifest at the level of the genome.

Introduction

The Linker of Nucleoskeleton and Cytoskeleton (LINC) is a complex of proteins spanning the Nuclear Envelope (NE), which consists of three core members: lamins, sad1 and UNC84 (SUN) proteins and nuclear envelope spectrin repeat proteins (nesprins). The primary role of the LINC complex is to provide structure for and to tether intranuclear structures such as heterochromatin to structural domains in the cytoplasm such as the actin cytoskeleton and microtubules (MTs). The existence of this complex allows the rapid transmission of signals via mechanical stimulation from peripheral domains of the cell such as the plasma membrane and cytoskeleton directly to the nucleus to enable rapid gene expression responses, as well as facilitate the structural integrity of the nucleus [1]. The LINC complex spans the nuclear membrane via SUN and nesprin family proteins [2]. SUN1 and SUN2 bind directly to lamin A at the inner nuclear membrane and are essential in anchoring the LINC complex. Lamin B1 on the other hand weakly interacts with SUN1 and SUN2 [3]. SUN1 is dependent on lamin A for nuclear anchoring [4] and SUN domains also span the lipid bilayer of the nuclear membrane, where they bind nesprin via KASH domains on the inner surface of the outer membrane [5]. Nesprins are transmembrane proteins that anchor to SUN in the nuclear envelope and project into the cytosol where they bind

directly to actin, MTs, intermediate filaments such as desmin, and centrosomal proteins (Figure 1) [6, 7]. Noncovalent interactions and disulfide bonding between nesprin and SUN resist mechanical force from the cytoskeleton and serve to maintain nuclear shape under stress [5]. Other NE membrane components of importance include LAP2, emerin and MAN1 (the so called LEM domain proteins), which interact with the core LINC proteins at the interface of the inner nuclear membrane and peripheral heterochromatin [8]. The importance of the LINC complex is highlighted by the spectrum and severity of diseases in which mutation to one of these core LINC members, most commonly lamin A/C, is causative [9-11]. Mutations commonly result in diseases of striated muscle i.e. muscular dystrophy and cardiomyopathy and many studies of mechanobiology have identified cell and molecular mechanisms, however the impact on regulation of the genome is still relatively poorly understood on a mechanistic level. Some recent findings, however, have expanded our understanding in this field. Herein we discuss some of the existing models of mechanobiology regarding the LINC complex, known links to genome regulation and reveal exciting new areas that require focus.

The mechanics of LINC complex misregulation in muscle disease

The family of lamin proteins consists of lamins A/C (A-type) and lamins B1 and B2 (B-type). B-type lamins arise from the genes *LMNB2* and *LMNB1* respectively, whereas lamin A and lamin C arise from the *LMNA* gene and occur by alternative splicing [12]. Lamin A arises via proteolytic processing of its precursor, prelamin A, by the zinc metalloproteinase ZMPSTE24 [13]. Accumulation of unprocessed prelamin A has been evidenced in genetically mediated laminopathies such as Hutchinson-Guilford Progeria Syndrome (HGPS), Emery-Dreifuss muscular dystrophy (EDMD) and dilated cardiomyopathy (DCM), whilst also accumulating in response to pharmacological inhibition of ZMPSTE24 [14-16].

Many disease-causing mutations have been described in lamins A/C but there is very little genotype-phenotype correlation and effects of single mutations are often pleiotropic, making mechanistic study challenging [17]. One explanation may lie in the role of the nuclear lamina (NL) in setting tissue stiffness. Central to the role of the NL is the ability to ‘set’ nuclear stiffness based on the external mechanical environment (cytoskeleton, extracellular matrix) and to modulate this in response to perturbations in mechanical loading. This was demonstrated elegantly by Swift and colleagues who showed that lamin A acts like a viscous fluid to impede nuclear movement under

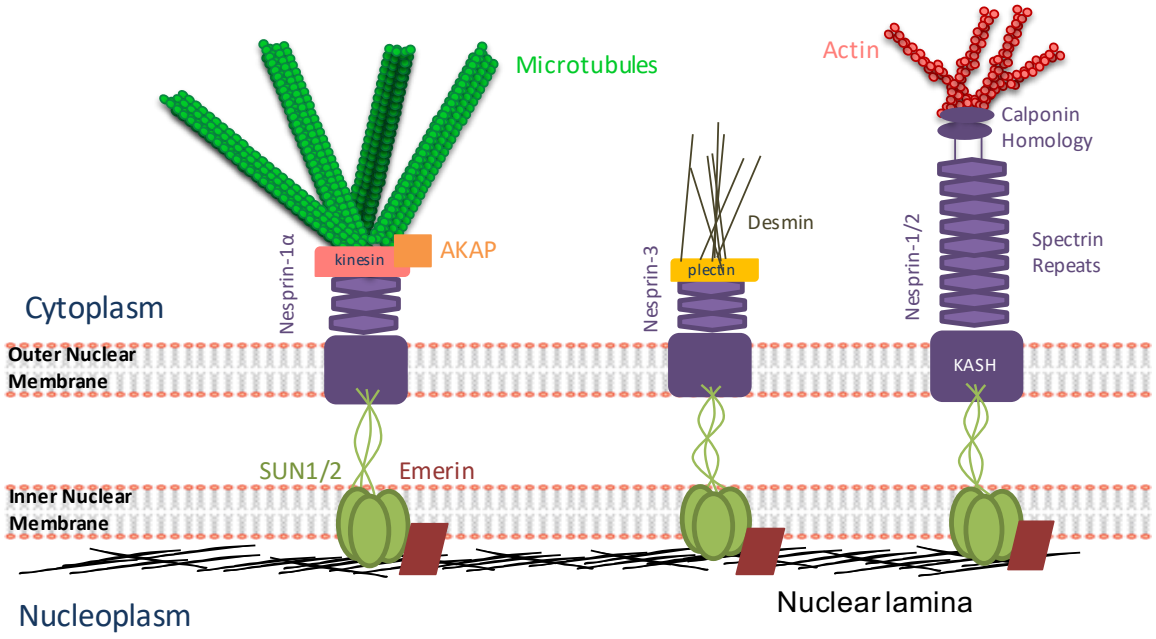

Figure 1: The Linkers of nucleoskeleton to cytoskeleton (LINC) complex in muscle cells. The LINC are a complex of proteins that interact to physically connect the nucleus to the cytoplasm. The nuclear lamina consists of A-type and C-type lamins localized on the inner nuclear membrane. The lamina is directly connected to SUN1, SUN2 and Emerin on the inner nuclear membrane. SUN proteins penetrate through the intermembrane space and bind nesprins on the outer nuclear membrane via the nesprin KASH domain. There are multiple nesprin isoforms, including Nesprin-1/2, Nesprin-1α and Nesprin-3. Nesprin-1/2 contain multiple spectrin repeats that connect the KASH domain to a calponin homology domain in the cytoplasm, which in turn binds actin. Nesprin 1-α interacts with microtubules in the cytoplasm via kinesin and other microtubule organising proteins i.e. AKAPs. Nesprin-3 binds cytoskeletal intermediate filament protein desmin via its plectin interating domain.

stress, whereas lamin B acts to return the nucleus to its original shape [18]. Nuclei from a range of cell types was aspirated in micropipettes to imitate tissue stiffness and real-time imaging was used to determine nuclear shape and lamin density. The authors describe the nuclei reacting in a viscoelastic manner much like a balloon, wherein viscosity describes the ability of the nucleus to impede deformation and elasticity describes the ability of the nucleus to return to its normal shape. The viscosity was increased compared to the elasticity in a lamin A: lamin B dependent manner. Fluorescence correlation spectroscopy showed that lamin A is the mobile component of the NL while lamin B is the immobile component. Additionally, mRNA analysis showed that the ratio of lamin A vs. lamin B in cells was determined by the mechanical environment where ‘stiff’ tissues, i.e. with an increased young’s modulus, result in cells with higher abundance of lamin A [19]. Additionally, a recent study has more detail on the biochemical and biophysical properties of lamin

A and revealed that under compression the coiled coils in the rod domains of lamin A polymers are able to slide onto each other to contract the length of the rod, behaving as a compression spring able to absorb pressure, supporting the idea that A type lamins can serve as a 'pressure valve' [20]. Disease causing mutations likely affect such properties. In one study the *LMNA*-S143P mutant, which causes familial DCM, led to lamin A proteins being more mobile and less tightly bound to the lamin network than wild-type (WT) proteins [21]. Lamin staining and confocal microscopy of DCM patient tissue showed that in the S143P mutant lamins were mislocalized to the nucleoplasm. The lack of lamin A incorporation into the NL resulted in aggregate formation that was unequally distributed around the nucleus and detrimental to the structural stability of the nucleus. Moreover, DCM mutations L85R and N195K and EDMD mutation L530P are known to diminish emerin-lamin interactions [22]. In another study, the ultrastructure of the NL with *LMNA* mutations E161K and R190W, exhibited lower cross-link density and increased network bundling. Functionally, reduced elasticity was observed making it more difficult for the cell to adapt to local stress [23]. Induction of mechanical stress has also been shown to result in nuclear strain changes and nuclear deformation in lamin A knockout (KO) fibroblasts [24] as well as in cells harbouring muscular dystrophy-causing *LMNA* mutations E358K and ΔK32 [25]. In summary, mutations in LINC proteins depletes the ability of the overall complex to maintain nuclear shape in the face of external stress.

Nesprins are encoded by *SYNE1* and *SYNE2* genes and occur in many isoforms [26]. The isoforms present in loaded tissue such as skeletal and cardiac muscle are nesprins 1α, 2α and 3. Nesprins are localized at the ONM where nesprin 1α and 2α bind MTs in the cytoskeleton via kinesin [27] and nesprin 3 interacts with actin via plectin and desmin domains [26, 28]. Investigations linking disease causing mutations in *SYNE1* to mechanical defects in striated muscle are limited compared with lamin A/C, but there is evidence that some are associated with EDMD and DCM [29]. Overexpression of the mutations R8272Q, S8381C and N8406K in human osteosarcoma cells was associated with abnormal nuclear morphology due to disrupted LINC complex interactions. Nesprin 1α2 anchors the nucleus to the MT motor protein kinesin during myotube formation, this interaction was disrupted and myonuclear position was disturbed in the mutated cells. Despite the rarity of these mutations in the DCM population, it is important to note their merit in describing the few clinical manifestations of laminopathies that are not associated with lamins.

Mutations in SUN1 and SUN2 are a much more subtly associated with disease than lamins and nesprins, are rare and may not be independently causal. Screening of SUN1/2 in EDMD patients revealed that SUN1/2 sequence changes were correlated with increased severity of the disease [30]. However, when compared to sequenced genome databases, these mutations were rare, which further complicates our understanding of their involvement. *LMNA* R453W EDMD is generally not associated with severe cardiac disease, however, in conjunction with SUN1 W337C mutation, resulted in early heart failure. This same study also showed that G68D, G338S and W377C SUN1 mutations, and A56P and R620C SUN2 mutations impaired rearward positioning of the nucleus due to defective centrosome orientation, with the interaction between SUN1 and emerin being impaired. Moreover, altered regulation of SUN1/2 has also been ascribed to the pathological progression of EDMD and HGPS, since SUN1 expression at the NE in cells from HGPS patients with the G608G and T623S mutations in *LMNA* was highly variable [31]. Supportive of this role in mechanotransduction via the LINC complex, SUN proteins have recently been identified as a modifier of mechanical stimulation within the 'outside-in' mechanotransduction pathway [32].

The role of the LINC complex in genome regulation

Gene expression responses to mechanical stimulation

In this section we outline the variety of evidence in multiple models pointing towards the idea that LINC protein expression and disruption serves to (dys)regulate the gene expression response to mechanical stress. The importance of the lamin network, in particular in potentially regulating gene expression, was evident in the early years of NE biology as disruption of this network was shown to cause chromatin leak into the nucleoplasm and chromatin loss in EDMD cells [33].

Other LINC components have subsequently been implicated in regulating gene expression in experiments showing that epitope-tagged SUN1-expressing cells grown on stiff substrate had enriched calcium ion (Ca^{2+}) transport, response to wound healing, cell adhesion, cell motility and extracellular matrix organization [34]. This study used the DAVID (Database for Annotation, Visualization and Integrated Discovery) assay to group the upregulated genes into functional classes. It was concluded that the LINC complex – via SUN1 – was directly related to mRNA expression of multiple genes for cytoskeletal, focal adhesion and NE elements.

Moreover, the LINC complex has been shown to regulate the genome in response to direct mechanical stimulation [35]. This study of mechanotransduction used magnetic tweezers with anti-nesprin-1 antibody to apply force on nesprin-1 of isolated nuclei. This triggered nuclear stiffening and the displacement of the magnetic beads was calculated to show that maintenance of nuclear shape was dependent upon an intact NL and emerin. It is well established that emerin interacts with the LINC complex via the lamina and phosphorylation of emerin at Y74 and Y95 by Src kinase facilitates interactions with lamin A/C. This interaction mediates a functional response to tension, presumed to be as a result of efficient gene expression programmes [36]. Indeed, lamin A deficient mouse embryonic fibroblasts with EDMD and DCM mutations exhibit more mobile and mislocalized emerin alongside actin polymerization defects which in turn impeded the nuclear localization of megakaryoblastic leukaemia 1, an important cardiac transcription factor [37].

In whole animal models, disruption to the LINC complex caused by lamin A depletion has been shown to disturb the cardiac hypertrophy response to a chronic pressure overload model and implicates impaired mechanotransduction signaling [38]. In this study, *Lmna*$^{+/-}$ mice were subjected to left ventricular pressure overload via transverse aortic constriction (TAC). Early growth response factor (Egr-1), a mechanosensitive gene, was downregulated in *Lmna*$^{+/-}$ cells compared to control resulting in accelerated heart failure, apparently bypassing the compensatory hypertrophy response. Post-TAC, the *Lmna*$^{+/-}$ cells displayed impaired nuclear mechanics and increased sensitivity to mechanical stress. As Egr-1 is also rapidly and transiently induced at the endothelial wound edge following acute aortic surgery, it is clear that Egr-1 is essential in cardiovascular tissue repair and its expression is dependent on the LINC complex [39]. The discrete mechanisms of how LINC complex components directly interact with DNA and chromatin to control gene expression have remained elusive. The NL is closely associated with chromatin and therefore its interaction is important in histone modifications such as acetylation and methylation – key components of transcriptional regulation [40]. Genomic interactions with the lamina occur at specific lamin associated domains (LADs) and are normally repressive in nature, but cause hyperacetylation in lamin A mutants [41]. Recently, compelling evidence has emerged regarding an epigenetic mechanism for altered cardiomyocyte contractility in iPSC cardiomyopcytes harbouring a K219T mutation in *LMNA* [42]. The basis of this mechanism was that mutant lamin binding to the promoter region of *SCN5A* gene, encoding the sodium channel Nav1.5, resulted in suppression of gene transcription via persistent co-binding of the polycomb

repressive complex 2 and tri methylated histone 3 lysine 27. CRISPR-Cas9 correction of this mutation relieved this repressive complex, restored *SCN5A* gene transcription and Nav1.5 protein abundance and sodium current density. This result supports the idea that the NE can regulate excitation in cardiomyocytes. Whether there is a mechano-regulatory element to this mechanism remains an unresolved question.

In summary, these results indicate a mechanism for mechanically stimulated changes in gene expression in the nuclear adaptation response to mechanical load. The interaction between various LINC components is essential in these responses, and precise mechanisms are starting to become more clear.

The role of muscle mechanobiology on genome integrity

Where regulation of the genome is concerned, the best characterised outcome of NE dysfunction in many cell types is genome instability whereby unrepaired DNA lesions accumulate [43]. This often results in the stalling of cell cycle and senescence in cells which are meant to undergo division. DNA damage and DNA repair are in fact a regular function within the realms of cellular homeostasis. Problems arise when the repair mechanisms become defunct and rates of repair are unable to match rates of damage, leading to accumulation of DNA lesions. Excess DNA damage results in expression of cell cycle inhibitors such as p16, p21 and p53, which cause cell cycle arrest and senescence [44]. Though cardiac myocytes are post-mitotic, they may still succumb to senescence by reducing protein turnover and cell repair. In fact, emerging evidence suggests that genome instability, brought about by loss of integrity to the nuclear envelope, may play an important role in the muscle cell function as these myocytes develop a number of signs of senescence. We recently investigated the role of cardiomyocyte-specific prelamin A accumulation in mice. We found that that mice developed rapid onset cardiomyopathy, which was underpinned by DNA damage accumulation, elevated senescence markers, and a profound inflammatory response [14]. Western blot analysis of LINC complex proteins found increases in an abundance of a number of LINC components such as nesprin-2 and SUN2, as well as the intermediate filament (IF) cytoskeletal protein desmin, which is known to link directly to the NE via the nesprin isoform nesprin-3. Indeed, another recent study has provided strong proof of the impact that IF and MT mechanics has on the integrity of the NE itself to reveal that nuclear shape is maintained and fine-tuned by a double-act of MTs and IFs [45]. Furthermore, this study confirmed the role of

mechanobiology in maintenance of the genome as DNA damage was elevated when the MT/IF ratio was increased. The authors were able to show gene expression changes involved in ion handling, contractility and mitochondrial function and metabolism occurred in cardiomyocytes with desmin knockdown. Finally, desmin depletion led to a considerable reduction in co-immunoprecipitation of chromatin LADs and lamin B1 compared to control myocytes indicating loss of heterochromatin regulation in these cells.

More recent evidence that molecular muscle mechanics regulates genome integrity has been provided by the Discher Lab who used the heart muscle of chick embryos to study the role of mechanics in NE and genome integrity [46, 47]. They showed that mechanically 'stiff' environments caused by increased extracellular matrix synthesis and high actomyosin contractility could lead to nuclear envelope rupture in cardiomyocytes, especially when lamin A abundance was low via lamin A knockdown. This led to a loss of DNA repair factors from the nucleus which in turn resulted in an elevation in DNA damage. Phosphorlyation of lamin A is known to induce its degradation to facilitate nuclear envelope breakdown for mitosis in cycling cells and accordingly baseline lamin A phosphorylation is low in muscle cells. Interestingly, this study also showed that inhibition of lamin phosphorylation by cyclin dependent kinases (CDK) prevented the mechanically regulated loss of lamin abundance. Functionally, DNA damage resulted in rhythmic changes to heart beating. Clinically, these data imply there is potential for CDK inhibitors to protect heart cell function in hearts which have undergone fibrotic disease remodelling. There is also recent evidence from experiments performed in skeletal muscle, that disruption to the lamina by disease causing lamin A/C mutations reduces the ability of nuclei to resist deformation caused by microfluid micropipetting and micro-harpooning experiments. This resulted in nuclear rupture, chromatin protrusions and persistent increases in DNA damage, which could be confirmed in muscle biopsies of patients exhibiting these mutations [48]. In non-muscle cell types, nuclear rupture has been shown to be a characteristic feature of lamin A/C deficiency and has been shown to result in uncoordinated exchange of nuclear and cytoskeletal components when fluorescently labelled in mouse and human fibroblasts [49]. It was observed that severe rupture events were coupled with resulting focal chromatin condensation likely affecting transcriptional regulation. Evidence suggests that nuclear rupture can cause dysfunctional gene expression as well as DNA damage responses. More work is required to determine whether these are co- or independent of each other.

LINC complex involvement in mechanically-regulated DNA replication

Tissue dynamics, which one might define as the role of cells and organelles in active tissues regulating their core functions (i.e. contraction in muscle) as well as growth and repair, are critically dependent on molecular events which serve to facilitate them. Whether by the synthesis of new proteins or generation of new cells by cell division in response to injury or stress, replication of DNA is likely important. Whether it can be regulated by direct mechanical modulation is a new question.

Mechano-transduction has been implicated in cell-cycle progression in the epicardial "wavefront" observed in a model of post-ischemic heart injury in transgenic zebrafish [50]. In this study, epicardial tissue was partially ablated and cellular morphology was examined by immunostaining of ZO1 – a tight junction marker. A 'cellular wavefront' was observed which was characterised by large multinucleate "leader cells" preceding small, mononucleate "follower cells". Live imaging concluded that defining features of the leader cells were increased stress fibres and endoreplication compared to the follower cells, which underwent cell division. The role of the leader cells was to migrate and maintain cell-cell adhesions whilst undergoing repair, for which endoreplication is necessary. Once repair was complete the leader cells underwent apoptosis and were replaced by follower cells. Since actin dependent migration was the source of leader cell stress, it was concluded that mechanotransduction played a role in leader cell cycle regulation. These findings are further supported by a recent study showing that lamin B2 is essential for progression to M phase, and may hold some control over the regenerative potential of cardiomyocytes [51]. In this study augmentation of lamin B2 expression, typically low in adult multinucleate polyploid cardiomyocytes, was shown to facilitate a reduction in ploidy, implicating reduced DNA replication in these cells. This facilitated nuclear envelope breakdown and metaphase transition, which in turn increased myocardial regeneration in mice and cell division in human cardiomyocytes from induced pluripotent stem cells. Moreover, the authors showed that zebrafish cardiomyocytes—typically regenerative—exhibited naturally high levels of lamin B2.

Though direct evidence for mechano-regulation is lacking in the aforementioned study on lamin B2, there is compelling evidence from *Drosophila melanogaster* (Fruit fly) muscle flank showing that the LINC complex plays a role in mechanically mediated DNA replication [52, 53].

Confocal microscopy showed that systematic disruption of LINC complex proteins klarsicht and karoid (*D.melanogaster* homologues of nesprin and SUN respectively) resulted in a small myonuclear size as the myonucleus did not grow synchronously with the myofibres themselves. However, analysis of Hoescht intensity revealed substantially increased DNA content indicative of polyploidy. The mutant cells underwent unchecked endoreplication during G1 and S phase of the cell cycle without progression to G2 and M phase. This was coupled with myonuclear positioning defects and highly varied nuclear size compared to myofibre size. Additionally, while control myofibres with an intact LINC slowed their DNA replication towards the end of development, mutant myonuclei persisted with endoreplication. Barrier to autointegration factor (BAF) is a key link between the NL and chromatin and was shown to be downregulated upon genetically-mediated disruption of klarsicht and karoid. Subsequent repression of BAF alone resulted in a similar phenotype suggesting BAF was the key link between the NL and proper cell cycle progression. Overall, these results indicate that an intact LINC complex supports cells in high-stress environments to maintain physiologic homeostasis i.e. growth and repair via regulation of DNA replication either directly or indirectly. Arguably more importantly, the intact LINC complex is essential in repressing endoreplication via BAF once growth and repair are resolved.

Overall, the data discussed in this section broadly support the idea that the NE is a mechanosensitive regulator of DNA replication. One question that remains to be answered unequivocally is whether the mechano-regulation of DNA replication actively contributes to DNA replication or passively regulates it through a scaffolding or structural role.

Roles for the LINC complex in mediating Ca^{2+} handling in myocytes

It has been recently emphasized that the integration of multiple signaling modalities (mechanical, soluble, ROS, ionic) is the 'true physiologic condition' and needs to be considered in approaches moving forward [54]. With this in mind Ca^{2+} signaling in the LINC complex field is desperately understudied. In this section, we will propose the role of LINC in the functions of Ca^{2+} for cardiac conduction, contraction and genome regulation.

The principle role of Ca^{2+} in excitation-contraction coupling in the heart is well defined [55], and there is some evidence to support a role for LINC complex proteins in regulating this. For example, HGPS patients display conduction defects in the form of repolarization abnormalities [56]. Electrocardiograms (ECG) of these patients revealed abnormal electrical activity of the heart

indicative of dysregulated Ca^{2+} handling between reticular stores and the cytoplasm known to negatively affect contraction and relaxation of cardiomyocytes. Supporting this, *Zmpste24*$^{-/-}$ mouse cardiomyocytes exhibited conduction defects due to reduced ability to maintain stable Ca^{2+} re-uptake because important Ca^{2+} reuptake proteins sarco/endoplasmic reticulum Ca^{2+}ATPase (SERCA) 2 and calsequestrin 1/2 were reduced. Contrary to this, *Lmna*$^{-/-}$ hearts showed no dysregulation in Ca^{2+} handling [57], whilst recently it has been shown that there are subtle changes to Ca^{2+} kinetics in pre-symptomatic cardiomyocytes from mice exhibiting the *LMNA* H222P mutation known to cause EDMD and DCM [58].

In 2007, Zima et al showed that the NE was a Ca^{2+} store and that the Ca^{2+} channels ryanodine receptors (RyRs) and inositol triphospate receptors (IP_3Rs) were abundantly expressed in the NE membranes [59]. It is well known that Ca^{2+}, in concert with CamKII-Calmodulin regulates functions of the genome in cardiomyocytes [60]. Therefore, one might hypothesise that LINC complex proteins may play a role in regulating the function and activity of these channels either indirectly by general NE disruption and spatial disorganisation, or directly by disruption to binding of complexes crucial to channel function. Indeed, immunofluorescence microscopy and co-immunoprecipitation has shown that the latter of these may be true since the muscle specific isoform nesprin-1α was shown to co-localize with muscle A-kinase anchoring protein (mAKAP) at the nuclear envelope in a model of neonatal rat cardiomyocytes [61]. This association was essential for the perinuclear localization of mAKAP and importantly, the ryanodine receptor (RyR) – a Ca^{2+} channel regulating efflux from perinuclear Ca^{2+} stores into the cytoplasm – was also precipitated with this protein complex. This result provides compelling evidence for the importance of the LINC complex in NE mediated Ca^{2+} signaling. These conclusions are however complicated by the finding that nesprin-1α has been shown to localize to the Z-disk of the sarcomere, meaning they may play a scaffolding role in regulating sarcoplasmic reticulum (SR) channels and/or myofilament activation in distal locations of the cell rather than at the NE [62, 63].

From the perspective of gene regulatory effects via Ca^{2+} handling, IP_3Rs are perhaps of greater interest since inhibiting RyRs in adult cardiomyocytes did not affect perinuclear Ca^{2+} spark frequency [59]. Moreover, IP_3Rs are mostly present within the inner nuclear membrane and regulate ion flow into the nucleoplasm. In ventricular cardiomyocytes, IP_3Rs are known to generate a nuclear Ca^{2+} signal responsible for transcriptional changes that regulate cardiac hypertrophy [64].

Subsequently Western blot analysis of human DCM samples showed increased IP_3R2 expression, suggesting hyperactive transcription via nuclear Ca^{2+} signaling may be augmented in heart disease. Additionally, ventricular myocytes treated with hypertrophy agonist endothelin-1 were shown to exhibit increased *Itpr2* expression, the gene encoding IP_3R2. Subsequently, fluorescent labeling indicated that NFATc, a hypertropy regulator, directly bound to the *Itpr2* promoter. Since calcineurin-NFATc signaling is a known pathway in cardiac hypertrophy, these results indicate that persistent nuclear Ca^{2+} sparks via increased IP_3R2 expression is a marked feature of pathologic heart remodeling [65]. Given the location of IP_3Rs in the NE and their role in gene transcription, it is reasonable to speculate that one of the LINC complex proteins are involved in regulating their function at the inner nuclear membrane (Figure 2A).

Importantly, there is also a precedent for mechanical regulation of Ca^{2+} in cardiomyocytes because physical transmission of stress from the sarcolemma to the SR via MTs has been shown to induce Ca^{2+} leak via the RyR2 [66]. In this study, carbon fibers attached to cell ends were used to induce stretch in rat ventricular myocytes and the Ca^{2+} spark rate was measured. Axial stretch caused an acute increase in Ca^{2+} only in the stretched part of the cell, implying a mechanical mechanism of Ca^{2+} release from the SR. In support of this finding, diastolic stretch in single mouse *mdx* myocytes generated transient increases in intracellular Ca^{2+} that propagated in waves, resulting in sarcoplasmic reticulum overload and sensitization of RyR2 channels [67]. Moreover, there is also evidence to suggest that the embryonic heartbeat is initiated by direct mechanical modulation of Ca^{2+} channels of the SR [68]. It is plausible that these mechanical mechanisms operating on the SR could do so in a similar manner at the NE and influence nuclear Ca^{2+} signaling and gene expression, which is likely to be defective in the face of LINC complex disruption. Based on the evidence presented, disruptions in various components that associate directly with MTs such as nesprin or indirectly such as SUN and lamin A are likely to play important roles in the pathological progression of cardiac disease associated with the nuclear Ca^{2+} transient (Figure 2B).

In terms of nuclear Ca^{2+} events during disease processes, studies have shown that, upon hypertrophic stimuli, Ca^{2+} is diffused from the nuclear membrane into the nucleoplasm and invagination is required for Ca^{2+} to be pumped back into the nucleoplasm [69]. Accumulation of perinuclear RyRs and SERCA after transthoracic aortic constriction in mouse hearts has also been observed and suggests that enhaced Ca^{2+} transport may be occurring at the NE and detrimentally affecting nuclear Ca^{2+} transients. This may suggest dysregulation, potentially augmentation, of

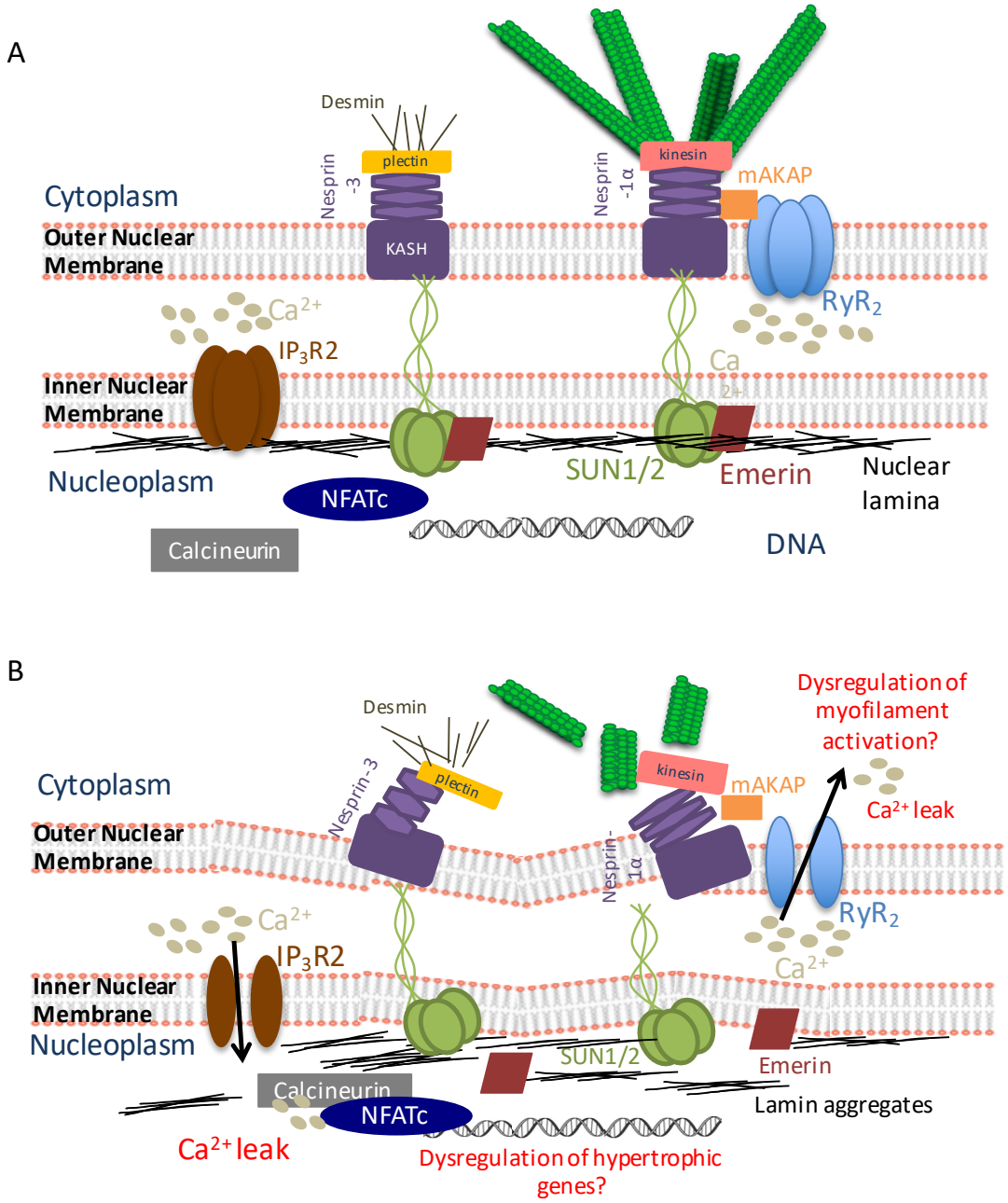

Figure 2: Hypothetical model of Ca^{2+} leakage from the transluminal space of the NE in LINC complex dysfunction. A. The space between the outer and inner nuclear membrane acts as a Ca^{2+} store. The IP_3R2 channel is located in the inner nuclear membrane and regulates Ca^{2+} movement into the nucleoplasm, while RyR_2 lies in the outer nuclear membrane and controls diffusion into the cytoplasm. **B.** A defective LINC complex may cause disruption of the IP_3R2 channel. Mishandling of Ca^{2+} transport into the nucleoplasm such as Ca^{2+} leak could lead to unregulated activation of the calcineurin-NFATc transcription factor complex. This complex directly binds DNA and promotes hypertrophic gene transcription. Disruption of the connection between the LINC complex and RyR_2 occurs via mAKAP and Nesprin-1α leading to Ca^{2+} leak into the cytoplasm and potentially contributing to perinuclear myofilament activation.

Ca^{2+} dependent NFAT and CaMKII signaling occurs that mediates hypertrophic gene expression programmes. There is early evidence to both refute and support the notion that the LINC complex could regulate the nuclear Ca^{2+} transient. Firstly, Muchir and colleagues have very recently published a study where they studied the amplitude of the nuclear Ca^{2+} transient in cardiomyocytes isolated from mice with a *LMNA* mutation known to cause EDMD and DCM in humans and found no difference compared to wildtype control mice at an early pre-symptomatic time point, suggesting that the LINC complex may not be all that important in regulating nuclear Ca^{2+} though it is important to note these experiments were performed in baseline conditions i.e. under no mechanical stress [58]. Indeed, hypertrophic stimulation of neonatal rat ventricular myocytes with emerin knockdown resulted in increased half-decay time of the nuclear Ca^{2+} transient, increased nuclear size and decreased nuclear invagination [70]. Emerin re-expression restored nuclear invagination, and increased nucleoplasmic Ca^{2+} transient. This result implicates emerin as a regulator of the nuclear Ca^{2+} transient, though it is not clear whether this is by direct association with nuclear Ca^{2+} channels or through general disruption of the NE. It is interesting that regulation of nuclear invagination formation and regression was attenuated in this study. On one hand, mechanical interplay between the cytoskeleton, NE and heterochromatin could regulate this, as it is now known that heterochromatin is also mechanically compliant and contributes to nucleus stiffness in non-muscle cell types (imagine a tug-of-war type scenario) [71, 72]. On the other hand there may be a regulatory role for the nuclear lamina in the *de novo* synthesis of phospholipid membrane to drive NE membrane extension as recently shown by experiments combining Nanoscale Secondary Ion Mass Spectrometry with Light microscopy that tracked nascent phospholipid formation and observed selective lamin incorporation during synthesis [73]. These processes may even be co-dependent.

Clearly much work is required to establish a role for the LINC complex and mechano mediated nuclear Ca^{2+} handling. Answering some of these questions could begin with investigating the consequences to the nuclear Ca^{2+} transient of mechanical strain alongside systematic removal of LINC complex components from myocytes. Moreover, defining a potential bio-physical and –chemical relationship between the Ca^{2+} channels such as RYRs and IP_3R2, with LINC complex components through contact prediction modelling, immunofluorescence colocalisation studies and co-immunoprecipitation will be important.

Conclusion

The current state of the LINC complex literature shows that components of the LINC complex act to convey mechanical pressure from the cytoskeleton in order to maintain nuclear shape, protect genome integrity with emerging data indicating that the LINC complex is able to help regulate dynamic events such as DNA replication and gene transcription. Disruption of the NE makes the nuclear membrane more susceptible to mechanical stress and leads to misregulation of the genome in multiple models of LINC complex disruption. In-depth mechanisms are still being elucidated but recent progress has been promising and the field is moving quickly.

The mechanobiology field is still quite new and understanding the relative contributions of mechanical versus chemical and electrical signaling is proving difficult to define. Indeed, improving our understanding of the position that the LINC complex occupies within integrated models of signaling, especially with regard to nuclear Ca^{2+} regulation, is required to provide a more complete picture of how mechanical stress elicits a (patho)physiological response. For example, Does the LINC complex regulate nuclear Ca^{2+} transient in skeletal and cardiac muscle cells under stress? If so, is this through direct mechanical modulation of local Ca^{2+} channels, or is this mostly a structural role i.e. maintaining an intact NE for stable membrane incorporation of channels? Can any observed effects be isolated from altered electrical or chemical modulation for a full reductionist understanding of these signalling realms?

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We declare no competing interests

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