Review Article

Unravelling the Connection: Investigating the Role of the LINC Complex in Ageing and Microgravity

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LAYMAN'S SUMMARY

The human body is continuously exposed to mechanical forces (Argentati et al., 2019). For example, when we walk, our bones and muscles experience the pressure of gravity. On a smaller scale, cells are also subjected to these forces, which play an important role in cell function and development. The ability to sense and respond to these mechanical stimuli is called mechanotransduction (Uzer et al., 2016). In simple terms, mechanotransduction is the process by which cells convert these mechanical forces into biochemical signals, which then trigger various reactions in the cell. Inside the cell, these forces and biochemical signals are carried from the cytoskeleton, which is a structural framework inside the cell just like our bodily skeleton, to the nucleus (Uzer et al., 2016). The nucleus is at the centre of the cell and is responsible for the storage of our genetic information (Gerace and Burke, 1988). The nucleus is surrounded by a protective barrier, called the nuclear envelope, and it has been shown that this nuclear membrane is important for a cell's overall function (Bouzid et al., 2019; Crisp et al., 2006). Moreover, it is important that the nucleus can respond to changes in the environment to determine cell shape and movement and adapt their gene expression patterns, which ultimately determines the cell's behaviour (Bouzid et al., 2019; Crisp et al., 2006). To be able to respond to mechanical forces, the nucleus is connected to the cytoskeleton via the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex (Crisp et al., 2006). The LINC complex consists of a group of different proteins which work together to connect the nuclear envelope to the cytoskeletal network of the cell. In this way, the LINC complex can transduce forces and biochemical signals from the cytoskeleton to the nucleus. Additionally, the LINC complex plays an important role in maintaining nuclear membrane integrity, which is important for regulating movement of the nucleus and to prevent damage to the DNA inside the nucleus, as defects to the LINC complex have been shown to be associated with muscle disease and ageing (Chang et al., 2019; Taranum et al., 2012).

In space, however, there is no to little gravity, which is called microgravity. Astronauts in space experience this microgravity as being weightless, and lack the resistance and mechanical forces that we experience on Earth. However, as a result of this, we observe that astronauts returning from space missions show different health problems, including bone loss and reduced muscle strength (Blaber et al., 2010). Therefore, scientists have become increasingly interested in understanding how loss of gravity affects the body, also on a cellular level, including the LINC complex and mechanotransduction. To our surprise, these health deficits from microgravity are quite similar to the effects that ageing has on the human body, which could mean that being in microgravity induces an ageing-like process (Blaber et al., 2010). However, this remains to be investigated. Therefore, this research aims to investigate the role of the LINC complex in ageing and in microgravity, to see whether loss of gravity indeed resembles an accelerated ageing process on a cellular levels. Firstly, the structure and the role of the LINC complex will be introduced. Then, the role of the LINC complex will be explored in ageing and microgravity. Finally, a comparison between the effects of ageing and microgravity on the LINC complex will be established. All in all, this review will summarize the current findings regarding the LINC complex in health and disease, and the importance of gravity in mechanotransduction, which will provide new insights in cell biology which can be used to improve the health of astronauts in space and in aged individuals on Earth.

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ABSTRACT

The Linker of Nucleoskeleton and Cytoskeleton (LINC) complex plays a crucial role in connecting the nuclear envelope to the cytoskeleton, providing structural support to the nucleus and facilitating mechanical signaling between the extracellular environment and the nucleus. Recent research in mechanobiology onboard the International Space Station (ISS) and in simulated microgravity (SMG) highlight the importance of gravity in functional mechanotransduction. Although most of the altered gravity research regarding mechanobiology has thus far been focussed on the cytoskeleton and the extracellular matrix (ECM), recent research demonstrates that SMG also induces changes in nuclear mechanics and gene expression patterns, which have been shown to be LINC complex dependent. Additionally, dysregulation of the LINC complex has been shown to disrupt nuclear integrity which leads to nuclear shape abnormalities in both Hutchinson-Gilford Progeria Syndrome (HGPS) and aged cells, which highlights the significance of the LINC complex and related proteins in ageing and age-related disorders. Interestingly, the microgravity environment seems to induce an accelerated ageing phenotype in astronauts, as the effects of spaceflight closely resemble those found in the elderly. Therefore, this review aims to explore the role of the LINC complex and related proteins in ageing and in microgravity, to further elucidate the interplay between loss of gravitational loading and ageing.

Keywords: *Linker of Nucleoskeleton and Cytoskeleton (LINC), Nucleus, Microgravity, Simulated microgravity (SMG), Ageing, Mechanotransduction, SUN1/2, Nesprins, Lamin A*

1. INTRODUCTION

In the field of cell biology, mechanotransduction refers to the process by which cells sense and respond to mechanical forces in their environment (Uzer et al., 2016). Cells, tissues, and organisms are continuously exposed to mechanical forces which emanate from various sources, such as shear stress, tension, compression, or substrate stiffness (Argentati et al., 2019). To effectively respond to such stimuli, cells have evolved sophisticated mechanisms to translate these mechanical cues into biochemical signals, which ultimately regulate essential cellular processes including gene expression, proliferation, differentiation, migration, and programmed cell death. Contrary to previous beliefs that considered the nucleus as a passive organelle exclusively responsible for storage of genetic material, emerging research points towards its active participation in sensing and responding to mechanical forces (Bouzid et al., 2019). As a result of these mechanosensing processes, structural alterations occur within the nuclear envelope affecting chromatin organization and gene expression (Uzer et al., 2016). One mechanism by which the nucleus participates in mechanotransduction is through the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex (Crisp et al., 2006). The LINC complex is composed of a group of proteins spanning the nuclear envelope that establishes a vital mechanical connection between the nucleus to the cytoskeleton. The LINC complex serves as a crucial intermediate for the transmission of mechanical signals from the cytoplasm to the nucleus. Moreover, the LINC complex has a pivotal role in defining nuclear architecture and positioning and regulates nuclear migration during cell division and differentiation (Crisp et al., 2006). Upon exposure to mechanical force, the LINC complex transduces mechanical and biochemical signals to the nucleus, leading to changes in nuclear morphology, chromatin organization, and transcriptional activity, which in turn regulate various cellular processes (Crisp et al., 2006). Defects in functional LINC complexes have been shown to disrupt nuclear envelop integrity which leads to nuclear shape aberrations in both Hutchinson-Gilford Progeria Syndrome (HGPS) and aged cells (Chang et al., 2019). Moreover, mutations in genes encoding components of the nuclear envelope and the LINC complex result in muscular dystrophy, heart disease, and various laminopathies, demonstrating an essential role of the LINC complex in maintaining nuclear envelope integrity (Taranum et al., 2012).

Recent research in mechanobiology onboard the International Space Station (ISS) and in simulated microgravity (SMG) demonstrate that functional mechanotransduction is heavily dependent on a critical physical force: gravity (Aventaggiato et al., 2020; Ogneva, 2022). As gravity is one of the factors under which life emerged and evolved, its absence is shown to have consequences on human health and performance, including bone mass loss, muscle atrophy, and cardiovascular problems (Blaber et al., 2010). Surprisingly, the effects of spaceflight closely resemble those found in aged individuals (Blaber et al., 2010). Although these effects are reversible once astronauts return to Earth, being in microgravity appears to induce an accelerated-

ageing phenotype. Microgravity has been demonstrated to affect the organization of the cytoskeleton and intracellular signaling, as well as differential expression of genes related to extracellular matrix (ECM), focal adhesions, and the cytoskeleton, particularly affecting cells of the musculoskeletal system and the heart which heavily rely on mechanical forces (Aventaggiato et al., 2020; Zhao et al., 2018). Recently, the role of gravity in nucleoskeleton-mediated mechanotransduction is explored, where simulated microgravity (SMG) has been shown to elicit changes in nuclear morphology and gene expression patterns, which have been shown to be LINC complex dependent (Neelam et al., 2020). Therefore, this review will explore the role of the LINC complex and related proteins in microgravity and its ageing-associated relevance, to investigate whether the observations in microgravity model an accelerated ageing process. If so, this will provide new insights in the fundamental, yet complex, mechanisms of nuclear biology and mechanotransduction, and its biological implications in microgravity, which might be used to improve the health of astronauts on long-term space missions.

2. THE STRUCTURE OF THE LINC COMPLEX

The nuclear envelope (NE) is a highly structured and regulated double-layered lipid membrane which separates the nucleus form the cytoplasm (Crisp et al., 2006; Gerace and Burke, 1988). It is composed of an outer nuclear membrane (ONM) and an inner nuclear membrane (INM), with a ~50 nm perinuclear space (PNS) that resides in between (Fig. 1). The ONM is continuous with the endoplasmic reticulum (ER), while the INM is connected to the nuclear periphery via the nuclear lamina. The nuclear lamina is a dense protein network consisting of type A lamins (lamin A and C) and type B lamins (lamin B1 and B2) which provide nuclear anchoring sites for the chromatin at the nuclear periphery. Moreover, aqueous channels spanning the two membranes, called nuclear pore complexes (NPCs), allow for the exchange of macromolecules between the nucleoplasm and cytoplasm. Together, the NE provides structural support, mediates interactions between the nucleus and the cytoplasm, and maintains proper genome organization.

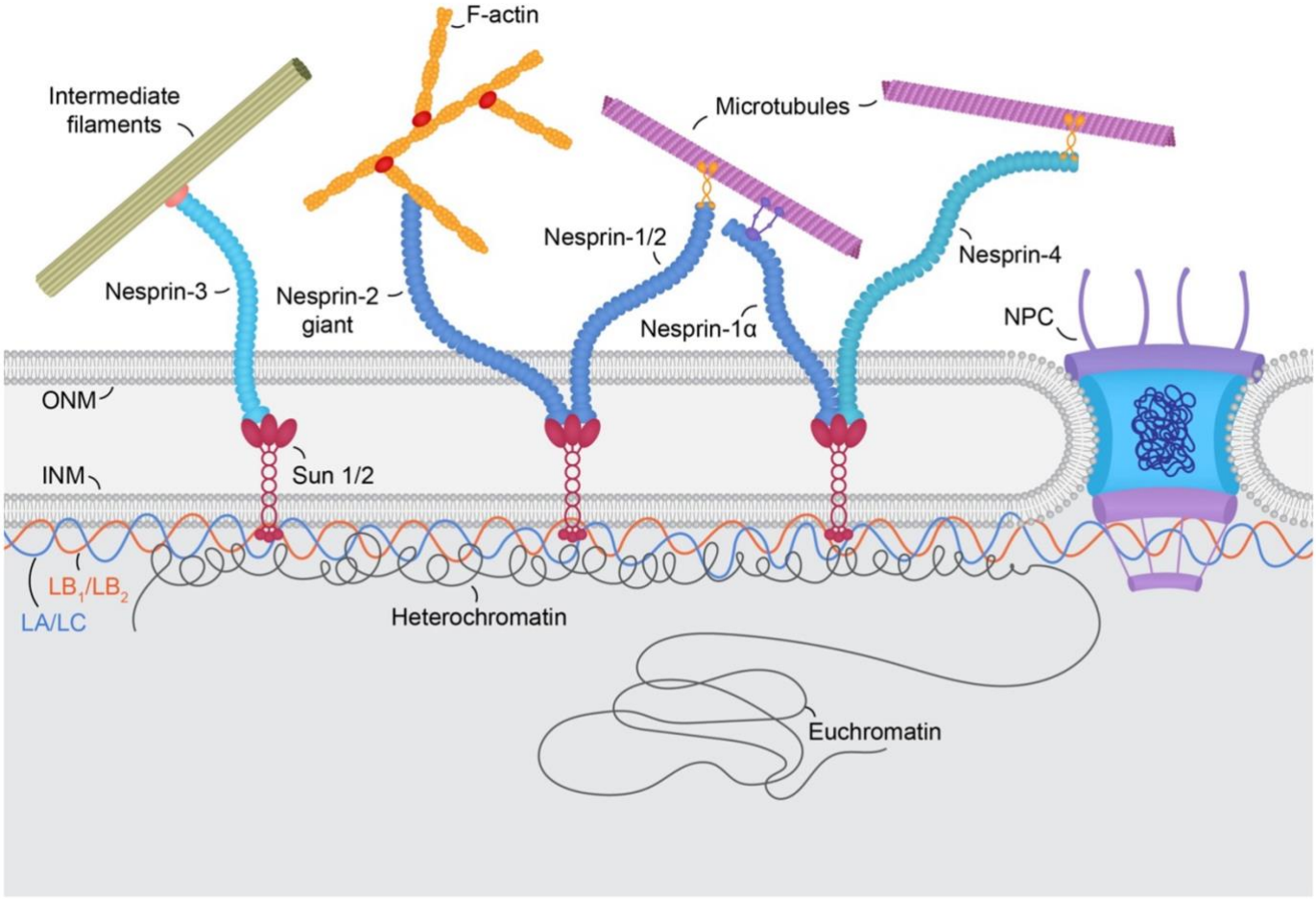

Fig. 1 – The LINC complex. The nuclear envelope (NE) is composed of an inner nuclear membrane (INM) and an outer nuclear membrane (ONM), with a perinuclear space (PNS) in between. Nuclear pore complexes span the INM and ONM and allow for exchange of macromolecules between the nucleus and the cytoplasm. The LINC complex connects the NE to the cytoskeleton. The LINC complex is composed of SUN and Nesprin proteins and associates with the INM and the ONM. SUN1 and SUN2 localize to the INM and interact with Nesprins, which reside in the ONM, in the PNS. The KASH domains of Nesprins interact with cytoskeletal elements in the cytoplasm. Nesprin-1 and Nesprin-2 couple microtubules and filamentous actin (F-actin), whereas Nesprin-3 binds intermediate filaments (IFs), and Nesprin-4 interacts with microtubules. Additionally, SUN1 and SUN2 interact with lamins type A/C (LA/LC) and type B (LB_1/LB_2) and Emerin (not shown) (Vahabikashi et al., 2022).

The LINC complex plays a critical role in connecting the NE to the cytoskeleton, providing structural support to the nucleus and facilitating mechanical signaling between the extracellular environment and the nucleus (Bouzid et al., 2019; Crisp et al., 2006). The LINC complex consists of two transmembrane proteins families: SUN (Sad1p and UNC-84) and KASH (Klarsicht/ANC-1/Syne Homology) (Fig. 1). The SUN proteins are INM proteins, of which SUN1 and SUN2 are ubiquitously expressed in mammalian cell types, that connect the INM to the nuclear lamina via lamin A, lamin C, lamin B1 and lamin B2. KASH domain proteins, called Nuclear envelope spectrin-repeat proteins (Nesprins), extend from the ONM into the cytoplasm and connect to the cytoskeleton via intermediate filaments (IFs), microtubules, and actin filaments. The C-terminal KASH domain of Nesprins and the SUN domain on SUN proteins interact within the PNS to form the LINC complex. Four different Nesprin genes have been identified, each having distinct cellular properties. Nesprin-1 and Nesprin-2 are the largest Nesprin proteins, often referred to as Giant Nesprin-1/2, and interact with microtubules and connect filamentous actin (F-actin) to the NE via their N-terminal calponin homology domain (CHD) (Warren et al., 2005). Specifically, Nesprin-2 has been reported to couple microtubules and actin via SUN1 and SUN2, respectively (Zhu et al., 2017). Nesprin-3 interacts with intermediate filaments (IFs) via plectins, and Nesprin-4 indirectly binds microtubules via kinesin (Roux et al., 2009; Wilhelmsen et al., 2005). Although full-length Nesprins reside in the ONM and interact with SUN1/2 in the PNS, shorter isoforms of Nesprins, like Nesprin-1α and Nesprin-2β, also localize to the INM to interact with lamins type A/C, SUN1, and Emerin, which all interact with one another to regulate nuclear shape and organization, cell signaling and gene expression (Mislow et al., 2002; Zhang et al., 2005). Therefore, different N-terminal domains and isoforms of Nesprins create a variety of LINC complexes and allow for the anchoring of different cytoskeletal components.

3. THE LINC COMPLEX IN NUCLEAR ORGANISATION AND FUNCTIONAL MECHANOSENSING

The LINC complex has a variety of roles in cellular organization. First of all, the LINC complex greatly contributes to the morphology, positioning, and orientation of the nucleus (Crisp et al., 2006). The shape of the nucleus is highly important for the integrity of the NE, cell migration and shape, the arrangement of chromosomes, and gene regulation, and defects in nuclear architecture are heavily associated with many disorders, including muscular dystrophy, cardiomyopathy, progeria, and cancer (Khatau et al., 2009). Recent research identifies the LINC complex as a key regulator of nuclear organization. Firstly, SUN1 and SUN2 have been shown to be essential for Nesprin-2 localization to the ONM (Crisp et al., 2006). Simultaneous knockdown of SUN1 and SUN2, and therefore uncoupling of Nesprin-2 from the NE, resulted in an increased PNS in HeLa cells. As a consequence, cells lost their nuclear integrity. In line with these observations, nuclear height was increased in lamin A/C-deficient cells (Vahabikashi et al., 2022). As lamins are connected to SUN1/2 in the INM and couple the nuclear membrane to actin caps, nuclear contractility was also reported to be lost. Similarly, depletion of Nesprin-1, which connects F-actin to the NE, results in heightened nuclei in endothelial cells and are no longer capable of reorientation in response to cyclic strain (Chancellory et al., 2010). Consequently, the observed increase in focal adhesions are suggested to compensate for the loss of tension between the nucleus and the actin cytoskeleton, as similar observations were made in myosin-inhibited cells (Chancellory et al., 2010). Moreover, the localization of Nesprin-2 to the NE is demonstrated to be an important regulator of migration and proliferation, as impairment of functional Nesprin-2 has been demonstrated to suppress nuclear motility as well as polarization of the nucleus, the latter resulting in disorganized centrosomes during cell division (Bouzid et al., 2019; Crisp et al., 2006). Finally, the LINC complex has an important role in maintaining genomic stability as LINC complex-dependent deformations of the nucleus have been shown to affect heterochromatin organization and show increased DNA damage upon stiffer nuclei (Vahabikashi et al., 2022). Collectively, these observations illustrate that an intact LINC complex is essential for a functional nucleus, and that the actin cytoskeleton connections seem to play an important role in mediating LINC complex-dependent nuclear motion and contractility.

Additionally, the LINC complex mediates the transmission of mechanical forces from the cytoskeleton to the nucleus, which is important for functional mechanotransduction (Uzer et al., 2016). There are multiple different types of mechanical forces that are exerted on cells, like shear stress, tension, and compression (Argentati et al., 2019). Apart from these external forces, internal mechanical cues, like actomyosin contraction and migration, are generated through adaptations and reorganization of the cytoskeleton. Under normal gravity, external stimuli are firstly perceived by the extracellular matrix (ECM), which is a network of fibrous proteins like collagen, elastin, and reticulin fibers, glycosaminoglycans, and proteoglycans, and provides structural support to cells (Stanton et al., 2019). The ECM transmits the mechanical force to the plasma membrane via focal adhesion (FA) complexes (Fig. 2) (Argentati et al., 2019). These FA complexes are composed of transmembrane integrin dimers which connect the ECM to the intracellular actin cytoskeleton via talin, vinculin, and paxillin, and focal adhesion kinases (FAKs). The mechanical force is converted into biochemical stimuli via focal adhesion kinase phosphorylation. Additionally, intracellular physical forces induce cytoskeletal remodeling via Rho A signaling and are transduced across the actin cytoskeleton to the nuclear lamina via the LINC complex. Consequently, both biochemical and mechanical signals can induce the expression of mechanoresponsive genes upon reaching the nucleus in order to adapt to physical changes in the environment (Uzer et al., 2016).

The LINC complex, therefore, is an important transducer of mechanical information. For example, the LINC complex is associated with the nuclear localization of Yes-associated protein (YAP) and β-catenin, which are important regulators of proliferation and stem cell maintenance (Shiu et al., 2018; Uzer et al., 2018). YAP/TAZ is a molecular sensor of mechanical force and is translocated to the nucleus upon changes in actin cytoskeleton tension (Booth et al., 2019). This nuclear translocation has been shown to be accompanied by the LINC complex, as disruption of the LINC complex through Nesprin-1 depletion resulted in decreased nuclear YAP in mesenchymal stem cells upon cell stretching (Bouzid et al., 2019). Similarly, loss of lamin A/C expression leads to decreased nuclear entry of YAP, indicating that transduction of mechanosensors from the cytoplasm to the nucleus is dependent on nucleocytoskeletal anchorage (Shiu et al., 2018). Interestingly, similar observations have been made for β-catenin. β-catenin, like YAP, is a transcriptional activator which accumulates in the nucleus upon mechanical stress, as mechanical cues prevent the degradation of β-catenin through FAK-activated Akt-mediated inhibition of GSK3β (Uzer et al., 2018). Detachment of the NE from the cytoskeleton through co-depletion of SUN1/2 was demonstrated to reduce nuclear trafficking of β-catenin, which further highlights the profound role for the LINC complex in mediating functional mechanotransduction.

Together, these findings demonstrate that the integrity of the LINC complex is highly dependent on SUN-KASH interactions and the nuclear lamina, and loss on either side results in disoriented and abnormal nuclei. As a consequence, this results in improper nuclear and cellular migration and inappropriate positioning and anchoring of chromosomes during cell division. Moreover, the LINC complex-mediated mechanical coupling of the nucleus to the actin cytoskeleton allows for functional mechanosensing of β-catenin, YAP/TAZ, and additional force-responsive signaling pathways, and demonstrate an active and crucial role of the LINC complex in mechanotransduction and subsequent physical adaptation to the cell's environment.

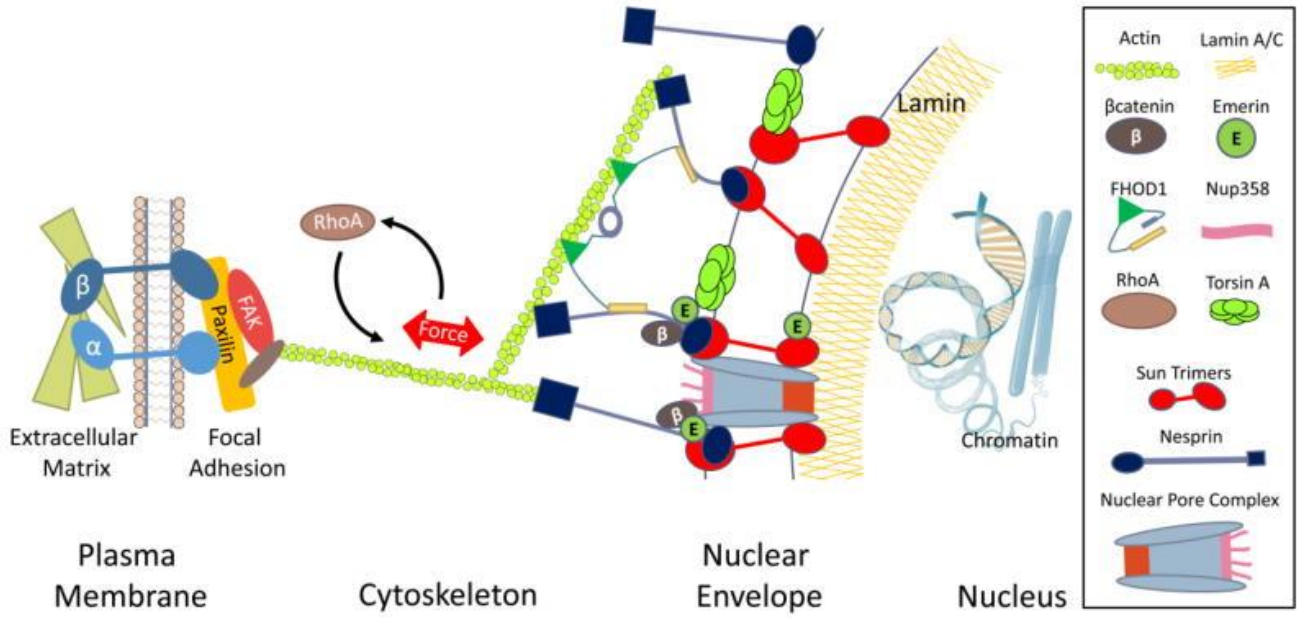

Fig. 2 – Mechanotransduction from the ECM to the nucleus. External mechanical cues are perceived by the extracellular matrix (ECM) and transmitted over the plasma membrane via integrins, which connect the ECM to focal adhesion (FA) complexes. The FA complexes are connected to the intracellular actin cytoskeleton and convert the mechanical signal into biochemical signals. Moreover, internal mechanical forces are transmitted across the actin cytoskeleton and induce RhoA-mediated remodeling of the actin cytoskeleton. Both mechanical and biochemical signals are transduced from the cytoskeleton to the nucleus via the LINC complex, which allows for nuclear mechanotransduction and induction of mechanosensitive genes (Uzer et al., 2016).

4. THE LINC COMPLEX IN AGEING AND DISEASE

As the LINC complex is an important intermediate of functional mechanotransduction between the cytoplasm and the nucleus and determines the physical architecture of the nucleus, dysfunctions in the LINC complex have been shown to be associated with disease, including ageing (Chang et al., 2019; Chen et al., 2012; Scaffidi and Misteli, 2006; Vidal et al., 2012). Ageing is a gradual, complex process which induces irreversible physiological changes over the course of time (Bajpai et al., 2021). These changes not only affect a wide range of tissues and organs, but also manifest itself at the cellular and molecular level. Particularly, ageing affects the mechanobiological properties of cells, permanently influencing the cell's ability to sense and adapt to changes in the cell's environment through changes in cytoskeletal features and mechanosensitive signalling pathways. The inability to sense and respond to inter- and intracellular forces results in ageing-related phenotypes like increased cell stiffness, decreased cell-plasticity, and a reduced

ability to cope with mechanical loading (Bajpai et al., 2021). Similarly, ageing has been associated with altered nuclear morphology, as aged cells display nuclear shape irregularities which are associated with increased levels of DNA damage (Bajpai et al., 2021). Consequently, the dysfunctions in the nucleocytoskeleton and subsequent mechanotransduction result in problems in the cardiovascular and musculoskeletal system, the skin, and the immune system. Collectively, altered nuclear mechanics and alterations in cytoskeletal components are indicative of altered LINC complex functioning during ageing.

4.1 The LINC complex in ageing and progeria

As the LINC complex plays a particular role in mediating nuclear mechanics and transducing mechanical stimuli, dysregulations within the LINC complex have been associated with ageing (Scaffidi and Misteli, 2006; Vidal et al., 2012). For example, aged cells display distorted nuclei compared to young cells which relate to changes in lamin A expression, including the expression of mutant lamin A isoforms (Scaffidi and Misteli, 2006; Vidal et al., 2012). However, the role of the LINC complex in natural ageing remains difficult to characterize, as ageing is a gradual, heterogenous, and long-term process. Alternatively, progeroid syndromes present a unique model system to study physiological changes in accelerated ageing.

One of the ageing-associated diseases in which the role of the LINC complex is explored is Hutchinson-Gilford Progeria Syndrome (HGPS), commonly known as Progeria (Chang et al., 2019). HGPS is an accelerated ageing disease caused by mutations in the *LMNA* gene, which encodes prelamin A, the precursor of mature lamin A, and lamin C (De Sandre-Giovannoli et al., 2003). In healthy cells, prelamin A is processed into mature lamin A via four modifications (Young et al., 2006). In HGPS, mutations in the *LMNA* gene induce a specific splice variant of prelamin A which lacks the necessary final cleavage site in prelamin A, resulting in mutant prelamin A which remains farnesylated, called progerin. As a result, progerin accumulates in the INM, which leads to misshapen nuclei and altered nuclear mechanics which induces severe tissue pathologies with drastically shortened lifespans (De Sandre-Giovannoli et al., 2003). As the underlying mechanisms behind the phenotype induced by progerin are not well understood, multiple studies have aimed to further define how the expression of progerin leads to premature ageing (Chang et al., 2019).

As aforementioned, lamin type A/C and type B are important interaction partners of the LINC complex and localize to the INM to anchor the chromatin to the NE (Crisp et al., 2006). Moreover, lamins interact with SUN1 and SUN2 and anchor the LINC complex to the INM. Lack of lamin A has been shown to result in dysfunctional LINC complexes, affecting nuclear integrity and cytoskeletal structures, likely via loss of lamin A-SUN interactions (Crisp et al., 2006; Vahabikashi et al., 2022). Indeed, research by Crispr et al. demonstrated a close association between the LINC complex and nuclear lamina abnormalities (Crisp et al., 2006). The authors used LMNA-deficient HeLa cells to investigate the relationship between SUN1 and SUN2 and lamin variants and demonstrated a specific preference of SUN1 protein for full-length prelamin A versus mature lamin A. This preference was absent for SUN2, which interacted with all four lamin types, although interactions with lamins B1 and C were weaker. Interestingly, introduction of farnesylated prelamin A resulted in loss of SUN2 from the nuclear lamina, whilst SUN1 remained unaffected. Similarly, research by Chen and colleagues demonstrated and increased affinity of SUN1 for farnesylated prelamin A and progerin, but not for unfarnesylated prelamin A (Chen et al., 2014). Moreover, the researchers demonstrate that progerin reduced the mobility of SUN1. Interestingly, Chen and co-authors further explored the association of the LINC complex and progerin, and found that SUN1 was overexpressed in the NE and at the Golgi in HGPS cells, which positively correlated with nuclear shape defects and cell death, which could be the result of reduced SUN1 mobility by progerin (Chen et al., 2012). Intriguingly, double $LMNA^{-/-}SUN1^{-/-}$ knockout mice showed a restored healthy phenotype, with increased proliferation rates, increased bone densities, reduced cardiac and muscle abnormalities, and a strongly prolonged lifespan. Moreover, inhibition of SUN1 in LMNA-deficient HGPS cells via disruption of microtubule organization using nocodazole, but not latrunculin B which disrupts the actin cytoskeleton, rescued cells from the HGPS phenotype. Similarly, depletion of SUN1 in HGPS cells via siRNA significantly reduced senescence-associated β-galactosidase levels (SA β-gal), which is a marker for cellular senescence, to the levels observed in control cells. This indicates that the absence of mature lamin A, and the subsequent overaccumulation of SUN1, contributes to altered nuclear mechanics and ageing via disbalances in LINC complex components.

Interestingly, Mattioli and co-authors show that SUN1 expression increases with time, as SUN1 expression was dramatically increased in ageing individuals compared to young individuals (Mattioli et al., 2011). Similarly, Chang et al. report similar LINC complex abnormalities in HGPS cells as cells under physiological ageing (Chang et al., 2019). Like previous research, Chang and colleagues demonstrate increased binding between SUN1 and progerin, but not between SUN2 and progerin. Moreover, the mobilities of SUN2, Nesprin-2, and Emerin were significantly decreased in fibroblasts from HGPS individuals, which resulted in disturbed nuclear movement, polarization, and centrosome orientation. The observed polarity defects are suggested to be caused by a transdominant inhibitory effect of SUN2-Nesprin-2 by overexpressed SUN1, disturbing proper actin cytoskeleton anchorage to the NE. Indeed, depletion of SUN1 rescued the actin- and SUN2-dependent nuclear positioning. Likewise, SUN1 was found to be significantly upregulated in fibroblasts from ages individuals, and showed similar nuclear defects, which could be rescued by SUN1 depletion. Strikingly, the use of dynein inhibitors rescued nuclear positioning and reorganized the actin

cytoskeleton in both aged and HGPS cells and suggest a microtubule-dependent progression of ageing. Therefore, the observed disturbances in nuclear morphology, mobility, and positioning, ultimately resulting in decreased proliferative capacity and chromosome aberrations during cell division, are likely the result of increased SUN1 expression rather than SUN1's affinity for mutant prelamin A variants. Research by Kim et al. support this hypothesis and demonstrate that the HGPS phenotype is ameliorated upon overexpression of non-functional KASH2, thereby occupying the SUN1 binding sites and reducing SUN1-Nesprin-2 binding, while also reducing the vascular disease that is associated with HGPS (Kim et al., 2018). Moreover, as SUN1 is demonstrated to accumulate over time and has previously been shown to bind microtubules specifically, its overexpression likely creates a disbalance in cytoskeleton elements which decreases nuclear integrity via a microtubule-dependent manner, although this underlying mechanism remains to be further elucidated.

As the LINC complex is affected upon ageing and results in nuclear defects and cytoskeletal rearrangements, it can be speculated that LINC-mediated mechanotransduction is also impacted by natural ageing. Indeed, mechanical force transmission has been shown to be highly disrupted in pathological (HGPS) and physiological ageing as a result of LINC complex-dependent increased nucleocytoskeletal stiffness (Bajpai et al., 2021; Booth et al., 2015). In terms of mechanotransduction, *LMNA*-mutant HGPS cells have been associated with decreased nuclear translocation of β-catenin (Hernandez et al., 2010). Similarly, recent research demonstrates that nuclear stiffness is inversely correlated to the nuclear translocation of YAP (Kishore Srivastava et al., 2023). Furthermore, decreased YAP signaling as a result of nuclear stiffness was associated with increased SA β-gal expression and reduced telomerase (hTERT) activity (Kishore Srivastava et al., 2023). This indicates that disturbed LINC complexes can induce cellular senescence via upregulation of SA β-gal as a result of reduced mechanotransduction of YAP. On the other hand, the mechanoresponsive Notch signaling pathway, which is a regulator of stem cell fate and differentiation, was found to be upregulated in HGPS fibroblasts (Scaffidi and Misteli, 2008). Upon induction of progerin in mesenchymal stem cells (MSCs), the MSCs display adult stem cell dysfunction as a result of induced pathological ageing, which could be the result of progerin-induced dysregulation of Notch signaling.

Overall, the LINC complex is affected in both physiological and pathological ageing in a similar way. Although further research is needed to elucidate the complex mechanisms by which the LINC complex is altered upon ageing, the overexpression of SUN1 seems to be the biggest contributor to dysfunctional LINC complexes. The upregulation of SUN1 increases binding of the microtubule cytoskeleton, likely resulting in an imbalanced nucleocytoskeleton which permanently affects mechanotransduction.

4.2 The LINC complex in cardiovascular and musculoskeletal disease

Apart from ageing, the LINC complex has also been associated with a multitude of diseases, especially affecting cells of the cardiovascular and musculoskeletal system (Méjat and Misteli, 2010; Park et al., 2009). These systems are particularly sensitive to mechanical forces and mechanotransduction as their primary function is to provide structural support, stability, enable movement of the body, and thus require the dynamic adaptation to physical demands.

Laminopathies, a group of diseases with mutations in the *LMNA gene*, other than HGPS, particularly emphasize the importance of the nuclear integrity in musculoskeletal, neuromuscular, and cardiovascular tissue (Taranum et al., 2012). One of the laminopathies in which the role of the LINC complex is explored is Emery-Dreifuss Muscular Dystrophy (EDMD) (Taranum et al., 2012). EDMD is a rare genetic muscle disease which primarily affects voluntary muscles and the heart. EDMD is characterized by mutations in genes encoding LINC complex components lamin A/C, Emerin, and Nesprin-1/2, each giving rise to different types of EDMD. Research by Taranum et al. demonstrated that all mutations contributed to altered nuclear morphology and mechanics, which is characteristic for laminopathies, and affected cell adhesion, migration, and induced cellular senescence via upregulation of SA β-gal (Taranum et al., 2012). Similarly, research by Park and co-authors demonstrate nuclear membrane irregularities in EDMD and in Limb-gride muscular dystrophy type 1B (LGMD1B), another *LMNA*-mutant disease (Park et al., 2009). Additionally, EDMD mice lacking mature lamin A demonstrated mislocalization of SUN2 and Nesprin-1, and disruptions of Nesprin-1 resulted in an EDMD-like phenotype in mice and have also been associated with cardiac disease (Méjat and Misteli, 2010). As a result, nuclei of muscle cells were incapable of proper positioning, which is essential for the coordinated expression of muscle-specific genes, enabling muscles to contract efficiently, and ensuring the structural integrity of musculoskeletal cells. Combined, this demonstrates a key role of the LINC complex in nuclear morphology and positioning, and hence, cardiac and muscle function.

5. THE LINC COMPLEX IN MICROGRAVITY

The importance of cellular integrity and mechanotransduction is further addressed by research performed in microgravity (Aventaggiato et al., 2020; Ogneva, 2022). Astronauts returning from long-term spaceflights often display a subset of health problems. These include reduced bone density, weakened muscles, cardiovascular problems, and disturbed functioning of the immune system (Blaber et al., 2010). On the cellular and molecular level, the absence of gravity is shown to induce cellular morphology changes, cytoskeletal rearrangements, and disturbed overall cell functioning, and highlight the importance of gravitational loading in mechanoresponsive tissue (Aventaggiato et al., 2020; Ogneva, 2022; Vorselen et al., 2014). In terms of the

mechanosensitive musculoskeletal systems, multiple studies have demonstrated decreased actin/myosin filaments resulting in sarcolemma disorganization, resulting in weakened muscle tissue (Aventaggiato et al., 2020). Moreover, osteoblasts and chondrocytes can respond to changes in stiffness of their extracellular environment. However, as a result of loss of mechanical stimuli in microgravity, osteoblast abundance is significantly decreased along with downregulations of bone tissue proteins that are necessary for bone maintenance (Aventaggiato et al., 2020). Regarding the cardiovascular system, endothelial cells under microgravity were reported to have disorganized cytoskeletal features, particularly in the NE, and demonstrated decreased actin expression through downregulation of β-actin (Carlsson et al., 2003). Although the effects of unloading can be recovered upon return to normal gravity on Earth, it remains important to investigate how cellular systems are affected in microgravity.

Evidence in real and simulated microgravity report deformations to the nucleus which have been associated with microgravity-induced changes to the cytoskeleton, pointing towards a potential role of the LINC complex in regulating nuclear shape and mechanics under microgravity (Carmeliet and Bouillon, 1999; Hughes-Fulford et al., 2006; Thapar et al., 1994; Zhang et al., 2017). For instance, early biomedical space research demonstrated alterations in nuclear and cellular morphologies with increased nuclear, cytoplasmic and overall cell areas in rodent osteoblasts, and showed altered cytoskeleton elements, including reduced actin stress fibers in spaceflight (Carmeliet and Bouillon, 1999; Thapar et al., 1994). Similarly, research by Corydon et al. demonstrated that the actin cytoskeleton, in particular, is affected in space flown cells (Corydon et al., 2016). For example, the actin cytoskeletal network was found to be highly disturbed only seconds into microgravity, including increasing filopodia- and lamellipodia-like features. In contrast, cells under hypergravity did not show signs of filopodia- and lamellipodia-like features. Moreover, research by Hughes-Fulford and co-workers reported osteoblast cellular and nuclear morphology changes during spaceflight, including rounding of cells and elongated nuclei with differences in nuclear texture compared to 1g ground controls (Hughes-Fulford et al., 2006). Indeed, previous work by Hughes-Fulford and co-authors reported decreases in nuclear sizes after four and five days of spaceflight (Hughes-Fulford and Lewis, 1996). Interestingly, the observed elongation of the nucleus due to the space environment is speculated to result from alterations to the microtubule cytoskeleton, as previous research by the same group could restore morphology changes by inhibiting microtubules using nocodazole (Hughes-Fulford and Lewis, 1996). More recently, the morphology changes and behaviour of the nucleus induced by microgravity are explored in relation to induced mechanical remodeling. For example, the orientation and position of the nucleus is shown to be affected upon changing gravity vectors, which results in flattening of the nucleus, which are directly linked to cytoskeletal remodeling (Zhang et al., 2017). Additionally, the effect of microgravity on nuclear mechanics is suggested to be flight duration-dependent, as the nuclear volume and area were increased after one to three minutes into spaceflight, whereas these parameters decreased after longer durations in microgravity in human macrophages (Thiel et al., 2019a). Nonetheless, Neelam et al. suggest that the elasticity of the nucleus upon reorientation and movement in response to applied force is maintained by vimentin, which is an intermediate filament protein which interacts with IFs, lamin A/C, and SUN linkages, suggesting a role of the LINC complex in nuclear mechanics upon changes in mechanical forces, including gravity (Neelam et al., 2015).

As previously described, the LINC complex is an important mediator of nuclear mechanics and mechanotransduction, and defects in LINC complex elements have been associated with a multitude of musculoskeletal and ageing-associated disease. Together with the cytoskeleton, focal adhesions, and the ECM it forms a critical network which allows for mechanosensing and subsequent mechanoadaptation (Uzer et al., 2016). As evidence demonstrates that the formation and coordination of these compartments is highly dependent on gravitational loading, the role of the LINC complex as a gravisensitive unit has only recently begun to be explored. The LINC complex was firstly studied in SMG by Zhao and co-authors, where microgravity was shown to inhibit the expression of proteins associated with the LINC complex in BL6-10 melanoma cells (Zhao et al., 2018). The researchers investigated whether SMG-induced apoptosis was dependent on mTORC1, NF-kB, and ERK1/2 signaling. To induce microgravity, the researchers used a 3D clinostat, which is a machine which continuously alters a sample's orientation, and therefore the gravity vector, which have been demonstrated to be well-suited devices to replicate the effects of real microgravity and are frequently employed to study the cellular cytoskeletal organization under weightlessness (Hoson et al., 1997). Interestingly, Zhao and colleagues observed altered nuclear positioning under induced microgravity and demonstrate that this is the result of downregulation of lamin A, Emerin, SUN1, and Nesprin-3 expression (Zhao et al., 2018). Moreover, the downregulation of these LINC complex-dependent proteins inhibited functional ERK1/2 signaling under SMG, but not under normal gravitational forces. Furthermore, Zhao and co-authors suggest that the apoptosis-induced morphological changes under SMG are dependent on rearrangements of the cytoskeleton via downregulation of actin, focal adhesions, and downstream FAK/RhoA signaling – which is a regulator of cell proliferation, differentiation, migration, and apoptosis via mTORC1/NF-kB signaling. Alternatively, the use of FAK/RhoA signaling agonists restored focal adhesions, nuclear positioning, and cytoskeletal integrity, and promoted cell survival under SMG. Combined, Zhao et al. demonstrate, for the first time, that LINC complex elements are associated with cytoskeletal and cellular morphology changes under SMG.

These observations are in line with the observations from Touchstone and co-authors, who explored the relationship between stem cell proliferation and gravity, with a specific focus on the LINC complex (Touchstone et al., 2019). The researchers used a clinostat-based model to investigate how the absence of mechanical loading affects MSCs. MSCs are the progenitor cells of endothelial cells, fibroblasts, osteoblasts, adipocytes, chondrocytes, muscle cells, and cardiomyocytes and have excellent regenerative potential (Aventaggiato et al., 2020). Importantly, gravitational force is known to be crucial for proper musculoskeletal and osteoblast functioning and has been shown to determine MSC cell fate, as loss of gravitational loading induces differentiation into the adipocyte direction, further reducing bone and muscle mass. Touchstone and colleagues report increased nuclear rounding with an increase in MSC nuclear height after 72 h of SMG (Touchstone et al., 2019). Moreover, a decrease in lamin A, SUN2 and Nesprin-2 expression, but not SUN1, was reported, demonstrating that the LINC complex is affected by changes in gravitational force. As previous research by the same group demonstrated that upregulation of LINC-associated elements increased MSC stiffness, the researchers opted to rescue the SMG phenotype by inducing mechanical force via low intensity vibrations (LIV) (Pongkitwitoon et al., 2016). Indeed, LIV was shown to activate mechanosignaling, although LIV failed to restore a normal MSC phenotype when SUN1 and SUN2 were co-depleted, indicating that LIV-induced cellular adaptations are dependent on the connectivity of the nucleus to the actin cytoskeleton (Touchstone et al., 2019). Similarly, the expression of LINC complex elements lamin A and SUN2 were moderately restored after exposing MSCs to LIV, further indicating that a functional LINC complex is dependent on gravity-induced mechanical cues. Moreover, Touchstone and colleagues demonstrate that decreased MSC proliferation was associated with decreased YAP signaling which could be restored by application of LIV, further demonstrating that there is a close relationship between a functional LINC complex and YAP-associated mechanosignaling. Later research demonstrates that the YAP-mechanosignaling of MSCs under SMG is not dependent on FAK signaling, further suggesting that nuclear YAP levels are LINC-complex dependent (Thompson et al., 2020). Indeed, previous research in MSCs revealed that destabilization of the LINC complex through Nesprin-1 knockdown reduced nuclear YAP via depolymerization of F-actin (Chen et al., 2016).

To further define the role of the LINC complex in response to microgravity, Neelam et al. also investigated the LINC complex under SMG (Neelam et al., 2020). Firstly, the researchers investigated the nuclear morphology of human breast epithelial cells under 3D clinostat-induced SMG. Short-term exposure to SMG (2 h) significantly altered nuclear shape and increased the nuclear height, while long-term exposure to SMG (20 h) reduced nuclear height, indicating an adaptational mechanism over time in SMG. Interestingly, short-term exposure of SMG on cells with disrupted Nesprin-2 NE localization represented insignificant differences in nuclear morphologies compared to cells that were not exposed to SMG, demonstrating that nuclear morphology changes upon SMG require functional LINC complexes. On the other hand, long-term exposure of SMG on cells with disrupted Nesprin-2 NE localization showed increased nuclear heights compared to cells with disrupted Nesprin-2 NE localization under normal gravitational loading. Additionally, Neelam and co-authors reported LINC-dependent differential gene expression changes in 1g vs. SMG (Neelam et al., 2020). Neelam and colleagues demonstrated a significant increase in genes related to focal adhesions and, like Zhao et al, a decrease in ECM genes and Nesprin-3 expression in long-term SMG exposed cells compared to short-term SMG exposed control cells. Moreover, the research report differential gene expression of genes related to the cytoskeleton (KRT85) and chemokines (CXCL3) between control cells vs. LINC-disrupted cells under SMG, indicating that the regulation of these genes depends on functional LINC complexes. Interestingly, the researchers also demonstrated increased LINC-dependent expression of cancer-associated genes under SMG, suggesting that the disruption of the LINC complex under SMG can activate cancer-associated signaling pathways. Taken together, these results suggest that the mechanosensitivity of cells is highly dependent on functional LINC complexes, i.e., SUN-Nesprin linkages, which support the hypothesis that disrupted LINC complexes affect mechanosignaling and downstream gene expression via morphological changes of the nucleus.

Although multiple studies regarding the LINC complex have been performed in SMG, the LINC complex remains unexplored in spaceflight. Yet, a few studies in real microgravity do touch upon LINC complex-related elements and provide further evidence that the LINC complex is altered upon loss of gravitational loading (Baio et al., 2018; Malhan et al., 2023). For example, cardiovascular progenitor cells that were cultured onboard the ISS demonstrated increased expression of genes associated with the cytoskeleton and LINC complex like VIM (Vimentin) and LMNA (lamin A) (Baio et al., 2018). Alternatively, mechanoresponsive YAP expression was decreased in neonatal, but not in adult cardiovascular progenitor cells during spaceflight, indicating that the adaptations to the microgravity environment might be based on cell type and regenerative potential. Additionally, recent research by Malhan et al. investigated gene expression changes in skeletal muscle from long spaceflight missions (Malhan et al., 2023). Interestingly, the researchers identified a wide range of differentially expressed genes and show that SUN2 is differentially expressed in a clock-dependent manner.

All in all, recent research demonstrates that the LINC complex is affected in microgravity, which highlights the importance of the LINC complex in nuclear mechanics and mechanotransduction. Yet, only a few studies have specifically investigated the LINC complex in SMG, and additional research is essential to further elucidate the role of the LINC complex in microgravity.

6. DISCUSSION

The LINC complex anchors the nucleus to the cytoskeleton, allowing for mechanosensing and functional force transduction across the two cellular structures (Crisp et al., 2006). The nucleus is a sensitive organelle and responds to changes in the environment by changing the nuclear shape and rearranges the chromatin to alter gene expression, of which recent research has identified the LINC complex as a critical mediator (Bouzid et al., 2019; Crisp et al., 2006). Defects in the LINC complex have been associated with loss of nuclear integrity and nucleoskeleton connections resulting in a variety of diseases, including muscular dystrophy, cardiomyopathy, cardiovascular disease, and ageing (Chang et al., 2019; Mattioli et al., 2011; Méjat and Misteli, 2010; Park et al., 2009).

The aim of this research was to investigate and evaluate how the LINC complex is affected in ageing and in microgravity, and whether the alterations in the LINC complex under microgravity resemble an accelerated-ageing phenotype. Although aberrations in nuclear shape, integrity, and plasticity and reduced mechanobiological properties are highly associated with ageing, the role of the LINC complex in ageing is not yet fully understood (Bajpai et al., 2021). Nevertheless, multiple studies demonstrate that the LINC complex is affected during ageing (Chang et al., 2019; Chen et al., 2012, 2014; Kishore Srivastava et al., 2023; Mattioli et al., 2011). Especially SUN1 expression is highly altered in physiological and pathological ageing, which seems to induce a disbalance in LINC complex components, thereby disturbing normal LINC functioning and functional coupling of the nucleus to the cytoplasm (Chang et al., 2019; Mattioli et al., 2011). SUN1 is demonstrated to have an increased preference for lamin A, especially for farnesylated premature lamin A, and its overexpression is highly associated with distorted and misshapen nuclei (Chen et al., 2012, 2014). The overexpression seems to result from accumulation of SUN1 over time, as SUN1 is also found to be overexpressed around the Golgi (Chen et al., 2014). Moreover, the stiffening of the nucleus itself is suggested to be associated with increased cellular senescence via dysregulations in YAP/TAZ signaling, which has been shown to be LINC complex dependent (Kishore Srivastava et al., 2023). Therefore, it seems likely that the accumulation of SUN1 over time stabilizes microtubule binding but reduces SUN-Nesprin actin filament coupling, resulting in an imbalance in LINC complex elements that are necessary for proper nucleocytoskeleton anchorage, as the rebalancing of the cytoskeleton was shown to ameliorate SUN1-mediated LINC complex defects (Chang et al., 2019; Chen et al., 2012). Consequently, this leads to nuclear aberrations, defects in nuclear positioning and migration, and disturbed mechanosignaling, which in turn are associated with increased senescence via upregulated SA-β-Gal expression, although additional research is necessary to further characterize the underlying mechanism.

In terms of microgravity, the LINC complex remains underexplored, and the current research does not seem to align with one another. For one, the LINC complex in microgravity is associated with downregulation of lamin A, Emerin, SUN1, and Nesprin-3 expression in breast epithelial cells (Zhao et al., 2018). Similarly, Neelam and co-authors reported a decrease in Nesprin-3 expression in cells that were long-term exposed to microgravity (Neelam et al., 2020). However, research by Touchstone et al. demonstrated a decrease in lamin A, SUN2 and Nesprin-2 expression, but not SUN1 and Nesprin-3, in MSCs (Touchstone et al., 2019). Alternatively, all studies report deformations to the nucleus, although Touchstone et al. report an increase in nuclear height after 72 h of SMG, while Neelam et al. demonstrate an increase in nuclear height after short-term exposure (2 h) but a decrease in nuclear height after long-term exposure (20 h). These differences in experimental outcomes can be the result of cell type specificity, experimental design, and differences in SMG exposure times, as these measures were not consistent across the different studies. Moreover, it is worth noting that the LINC complex-dependent effects under microgravity can differ between primary and non-transformed cells versus transformed cell lines, as cancer is often already associated with a loss of expression of multiple LINC complex elements, including lamin A/C, SUN1, SUN2, and Nesprin-2 (Bouzid et al., 2019). For instance, and contrary to the observations in ageing, SUN1 expression is highly reduced in breast cancer tissue (Matsumoto et al., 2016). More importantly, malignant cells often already present nuclear abnormalities under normal gravitational loading, which could negatively impact LINC complex-related studies in microgravity, and alternatively, could give different outcomes than non-transformed cells under microgravity, since previous work identified different cellular behaviours of transformed and non-transformed T lymphocytes under microgravity (Cuccarolo et al., 2010; Singh and Lele, 2022). Therefore, the use of different non-transformed and transformed cell lines might explain some of the LINC complex-dependent differences in observations between these microgravity-related studies. As such, it is important that microgravity-related research is to be performed in primary and non-transformed cells, which could better model the physiological effects of microgravity in astronauts and, ultimately, improve astronaut health. Interestingly, the adaptations to the LINC complex in the absence of gravitational loading can be reversed via induction of LIV, although LIV could not restore functional mechanotransduction in SUN1 and SUN2 co-depleted MSCs under SMG, indicating that mechanotransduction depends on functional LINC complexes and mechanical loading (Touchstone et al., 2019). Similarly, LINC complex-dependent decreased nuclear YAP levels under microgravity could be restored using LIV, suggesting that induced mechanical loading can reverse the effects of microgravity, which supports vibration therapy as a potential mechanism for astronauts to stay healthy in space. However, the adaptations to the LINC complex remain to be further elucidated in real microgravity, as results from clinostat-based models do

not fully represent actual spaceflight conditions (Hoson et al., 1997).

Unfortunately, due to a lack of experimental output a true comparison of the LINC complex in ageing and microgravity cannot yet be established. Nonetheless, the LINC complex appears affected in both ageing and in microgravity, which is likely the result of changes in expression and localization of LINC complex elements. Consequently, SUN-Nesprin linkages are affected which alter nuclear coupling to actin filaments and microtubules. In ageing, this seems to be induced by accumulation of SUN1, leading to increased microtubule linkage via SUN1-Nesprin-2, thereby reducing F-actin coupling. As the actin cytoskeleton is particularly important for transduction of mechanical forces, the observed decrease in actin in aged cells likely hampers functional mechanotransduction in a LINC complex dependent manner. Although this potential underlying mechanism requires additional research, the downregulation of actin fibers is also observed in microgravity. Indeed, microgravity studies investigating the cytoskeleton report a downregulation of cytoskeleton components and demonstrate disorganized actin en microtubules (Janmaleki et al., 2016; Mao et al., 2016). However, it remains to be investigated how the LINC complex and its association with the cytoskeleton regulates nuclear and cellular morphology, stiffness, and resistance to mechanical forces under microgravity, as differences in cytoskeletal stiffness have been observed, where microgravity appears to increase cellular stiffness in MSCs but reduces stiffness in endothelial cells (Janmaleki et al., 2016; Mao et al., 2016). Finally, nuclear entry of YAP is decreased as a result of dysfunctional LINC complexes in both ageing and in SMG, which suggest that the microgravity-induced effects to the LINC complex might induce ageing-like phenotypes, although additional research is needed to confirm this hypothesis.

It is, for the reasons listed above, important that future research focusses on identifying changes in expression and localization of LINC complex elements in ageing and microgravity. Furthermore, as LINC complexes are not uniform but can establish different complexes via the expression of different Nesprin isoforms, it is crucial to investigate potential redundant mechanisms within LINC complexes under normal gravitational loading, microgravity, and in disease and ageing. Even though Nesprin-2 seems to be a dominant player in LINC complex functionality, as its uncoupling from the NE, directly or indirectly via loss of SUN1/2, results in nuclear defects affecting nuclear and cellular mechanics, the roles of its counterparts in nuclear integrity and cytoskeletal coupling also need to be further explored. Furthermore, it remains essential to investigate nuclear morphology changes, and the effects of dysfunctional LINC complexes on chromatin organization, proliferation, differentiation, and migration. To elucidate these LINC complex-related mechanisms in space, nuclear dynamics can be explored using the high-resolution FLUMIAS-DEA microscope, which can perform three-dimensional life cell imaging onboard the ISS to investigate LINC complex-dependent nuclear dynamics under real microgravity (Thiel et al., 2019b). Additionally, the FLUMIAS-DEA microscope can also be utilized to identify the impact of microgravity (~ 0g), hypogravity (< 1g), normal gravity (1g), and hypergravity (> 1g) on nuclear morphology, positioning, and migration and additional LINC complex-related processes using BIOPACK, which provides experimental gravity profiles ranging from 0.1g to 2.0g (van Loon, 2004). Lastly, the rebalancing of cytoskeletal components seems to rescue the LINC complex-related disease and ageing phenotypes, which suggests important roles of the LINC complex in cytoskeleton organization and dynamics and should be further explored. In conclusion, elucidating the role of the LINC complex in health and disease and its purpose as a gravisensitive complex will improve the current knowledge gap. These new findings will increase our understanding of nuclear mechanics and mechanobiology, which will be useful for the development of therapeutics for LINC complex-related diseases and ageing and can be used to better understand and ultimately reduce the health deficits in astronauts and future space tourists.

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