Chapter 9
The Nuclear Lamina: From Mechanosensing in Differentiation to Cancer Cell Migration

Jerome Irianto, Irena L. Ivanovska, Joe Swift, and Dennis E. Discher

Abstract How cells respond to physical cues in order to meet and withstand the physical demands of their immediate surroundings has been of great interest for many years, with current efforts focused on mechanisms that transduce signals into gene expression. Pathways that mechano-regulate entry of transcription factors into the cell nucleus are emerging, and our most recent studies suggest that mechanical properties of the nucleus itself are actively controlled in response to matrix elasticity in mature, injured, and developing tissue. Here, we discuss the mechano-responsive properties of nuclei as determined by intermediate filament lamin proteins that line the inside of the nuclear envelope and that also impact transcription factor entry and broader epigenetic mechanisms. We summarize signaling pathways that regulate lamin levels and decisions of cell fate in response to matrix mechanics combined with molecular cues. We also discuss recent work that highlights the importance of nuclear mechanics in niche anchorage and cell motility in development, hematopoietic differentiation, and cancer invasion whilst also emphasizing a role in protecting chromatin from stress-induced damage.

Keywords Cell mechanics • Mechanotransduction • Extracellular matrix • Nucleus • Nucleoskeleton • Proteostasis • Lamina • Differentiation • Cancer

9.1 Introduction

Mature tissues need to be particularly resistant to the mechanical demands of an active life. Our bones, cartilage, skeletal muscle, and heart tissues are stiff, making them robust to routine physical exertion such as walking or running when they are subjected to high-frequency shocks, stresses, and strains. With every heartbeat, the

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left ventricular wall experiences a 20 % radial strain (Aletras et al. 1999), and local strains of ~20 % also occur in the cartilage of knee-joints with every step (Guilak et al. 1995). Tissue-level deformations might even be amplified within cells and their nuclei (Henderson et al. 2013). Our softer tissues have less need for robustness because their function does not require them to bear load. Furthermore, some of our softest tissues, such as brain and marrow, are protected from an otherwise hard world by our bones. Nonetheless, when soft tissues are subjected to impact, such as a collision of heads in American football or rugby, occurrences of rapid straining can cause lasting damage (Viano et al. 2005).

We have recently sought to characterize the composition of cells and extracellular matrix (ECM) in tissues of increasing stiffness, and by implication, in tissues that are subjected to the greatest stress (Swift et al. 2013a, b). A close correlation between the concentration of ECM components and bulk tissue elasticity was discovered. More surprisingly, we also discovered a systematic scaling between tissue elasticity and concentration of lamins in the nucleoskeleton that was partially recapitulated in cultured cell systems. Corresponding changes in the mechanical properties of the nuclei suggest that this response may act to protect the precious chromatin cargo of the nucleus from shocks that are transmitted through the surroundings, across the cytoskeleton, and into the nucleus. An active regulation of cell or matrix composition in response to the environment implies feedback into pathways of protein turnover and remodeling, or control of the rate of new protein production. Responsive matching of mechanical properties to physical demands has classically been described as a "mechanostat" in the context of bone regulation (Frost 1987), but a recent explosion in mechanobiological studies has uncovered a host of other mechanically sensitive cellular phenomena, including contraction (Discher et al. 2005), migration (Hadjipanayi et al. 2009a, b; Winer et al. 2009), proliferation (Lo et al. 2000; Hadjipanayi et al. 2009a, b; Klein et al. 2009), differentiation (Engler et al. 2004, 2006), and apoptosis (Wang et al. 2000). Despite the recent progress, questions of how mechanical signals are transduced into specific transcriptional or regulatory pathways continue to challenge the field.

The lamina is a network structure formed from intermediate filament (IF) lamin proteins that lies inside the nuclear envelope and interacts with both chromatin and the cytoskeleton (Fig. 9.1a). In the somatic cells of humans, mice, and most vertebrates, the major forms of lamin protein are expressed from three genes: lamins -A and -C are alternative splicing products of the *LMNA* gene (collectively "A-type" lamins); lamins -B1 and -B2 are encoded by *LMNB1* and *LMNB2* genes, respectively ("B-type" lamins). The lamins share structural features, and indeed have some commonality in amino acid sequence, but differ in their posttranslational modification, with B-type lamins permanently appended by a farnesyl group that is cleaved from mature lamin-A (reviewed by Dechat et al. 2010). Like other IFs, such as keratin and vimentin, the lamins form coiled-coil parallel dimers that assemble into higher-order filamentous structures which fulfill important structural roles (Herrmann et al. 2009).

Here we aim to summarize recent efforts to characterize the proteins that vary systematically with tissue stiffness. The effects of the composition of lamina on nuclear mechanical properties will be elaborated in detail, and we will consider the

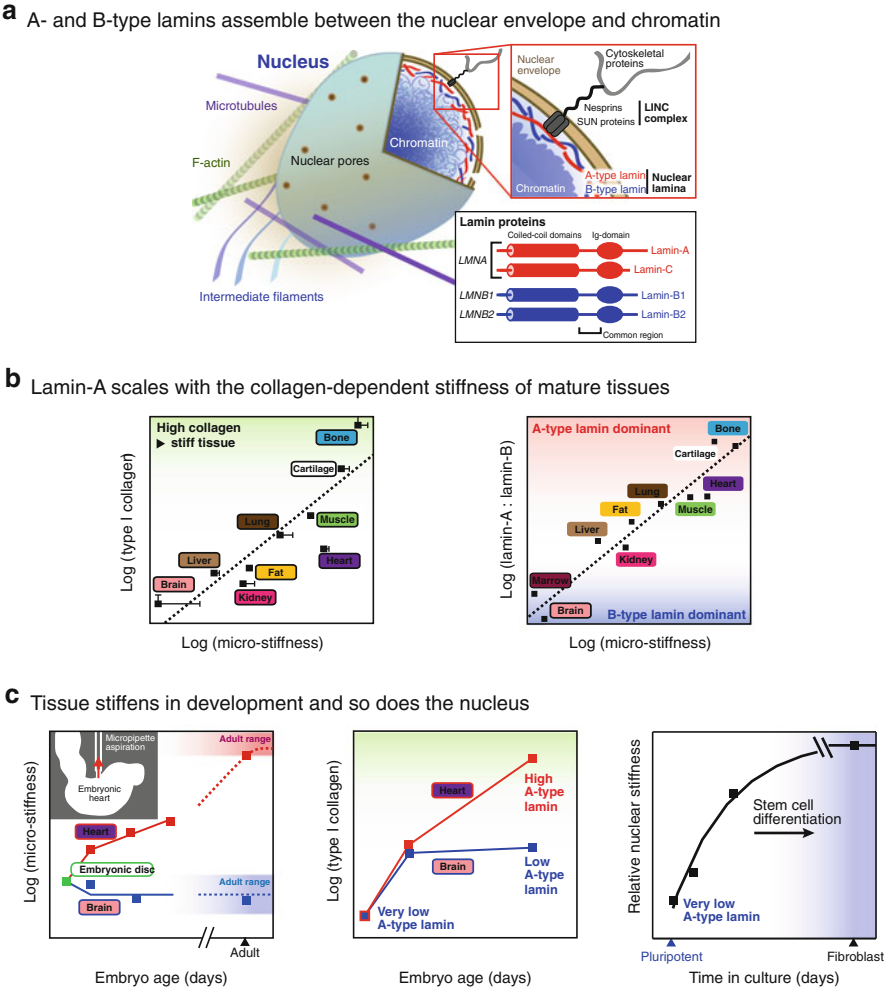

Fig. 9.1 Scaling of matrix and lamin in mature tissue and during development. (**a**) A-type and B-type lamins form juxtaposed networks on the inside of the nuclear envelope; they are effectively located at an interface between chromatin and the cytoskeleton, to which the lamina is attached through the "LINC" (linker of nucleo- and cytoskeleton) complex. "A-type lamins," lamins -A and -C are alternative spliceoform products of the *LMNA* gene; "B-type lamins," lamins -B1 and -B2 are protein products of *LMNB1* and *LMNB2*, respectively (adapted from ©Buxboim et al. 2010, originally published in The Journal of Cell Science). ((**b**)—**left**) The quantity of collagen-1 present in tissues scales with tissue micro-elasticity (Swift et al. 2013a, b). As collagen is one of the most prevalent proteins in the body, it is perhaps expected that it defines mechanical properties. ((**b**)—**right**) The composition of the nuclear lamina scales with tissue microelasticity. A-type lamins dominated the lamina in stiff tissue, whereas B-type lamins are prevalent in soft tissue (Swift et al. 2013a, b). ((**c**)—**left**) Observations made in adult tissue were also reflected in developing chick: the embryonic disc was initially soft, but divergent tissues either remained soft, such as brain, or became increasingly stiff, such as heart. *Inset*: developing chick hearts were probed by micropipette aspiration to determine micro-elasticity. ((**c**)—**center**) Tissue stiffening during development is accompanied by increased levels of collagen and A-type lamins (Lehner et al. 1987; Majkut et al. 2013). ((**c**)—**right**) Embryonic stem cells initially have negligible quantities of A-type lamins, but these levels increase as the nucleus stiffens during lineage commitment (Pajerowski et al. 2007)

functions of the lamina in transducing mechanical signals from matrix and surroundings into cellular response, both in terms of an active regulation of the lamina itself and its broader role as a linkage in mechanotransduction pathways. Although we focus on a primarily protective purpose of lamin in the nucleus, there are additional regulatory consequences of such a stiff and bulky organelle, and we will summarize recent evidence that such properties limit the freedom of cells to move through tissue. The proximity of the lamina to heterochromatin within the nucleus has led it to be widely associated with epigenetic regulation (e.g., Kim et al. 2011; Meuleman et al. 2013). This review will seek to highlight the pervasive influence of the mechanical role of the lamina and hence proposes that lamin acts as both guardian and gatekeeper for chromatin.

9.2 Scaling of ECM and Lamina Components in Mature and Developing Tissue

Collagens and other protein constituents of the ECM are the most prevalent proteins in our bodies, largely determining the mechanical properties of tissue. Collagens are found at higher levels in stiff, mature tissues where, consistent with an expectation for proteins to behave as "biological polymers" (Gardel et al. 2004), their increased concentration is the basis of tissue elasticity (Fig. 9.1b—left). By using quantitative label-free mass spectrometry (MS) for proteomic profiling (Swift et al. 2013a, b), we have shown that collagens and other ECM-associated proteins scale with tissue elasticity (Swift et al. 2013a, b). MS was also used to quantify roughly 100 of the most abundant proteins in the cytoskeleton and nucleus, and we found the strongest correlation with bulk tissue elasticity in the composition of the nuclear lamina (Fig. 9.1b—right). Although primarily characterized by the ratio between the two main families of lamins, A-type and B-type, the compositional scaling is dominated by a 30-fold increase in the concentration of lamin-A, C from brain to bone. Although our recent observations are broadly in agreement with an extensive literature in lamin quantification (e.g., Krohne et al. 1981; Rober et al. 1990; Cance et al. 1992; Broers et al. 1997), they provide a new perspective on systematic variations across many tissues.

The relationship between tissue stiffness, ECM, and lamina during development was also determined; micropipette aspiration of embryonic chick tissue showed that the homogeneous embryonic disc is initially very soft, with proteomic profiles indicating correspondingly low levels of collagen (Fig. 9.1c—left and center). However, the properties of different tissues diverge during development with the brain remaining soft, whereas the heart stiffens as ECM proteins are deposited (Majkut et al. 2013). Cells in stiffening tissues, such as heart, are also likely to have respectively higher levels of lamin-A,C (Lehner et al. 1987). Nuclei in embryonic stem cells have indeed been shown to be very soft and to have low levels of lamin-A,C (Pajerowski et al. 2007; Eckersley-Maslin et al. 2013). As these cells commit to a

lineage-specific fate, the levels of lamin-A,C increase and the nucleus becomes correspondingly stiffer (Fig. 9.1c—right).

Importantly, despite an apparent role in amplifying decisions of animal cell fate in conjunction with matrix elasticity, lamin-A,C is not essential to development as knockout mice still form all tissues (Sullivan et al. 1999). Likewise, lamin-B knockout mice survive embryogenesis (Kim et al. 2011). The most critical role of lamin may therefore be to tune the properties and regulation of maturing tissues in higher organisms, and its absence can perhaps be compensated for during development. However, the distinction here may be blurred: there is still a need to understand nuclear structure during some stages of development, such as during cell migration; and processes of trafficking and differentiation continue throughout an organism's life span. Nonetheless, there appears to be consistency with the current notion that lamins are not expressed in yeast and plants (Dittmer and Misteli 2011), despite the latter possessing genomes that are larger and more complex than those in animals. It seems very likely that the hard cell walls of these organisms protect the chromatin in ways that are not possible for animal cells with soft cell membranes. Cell biologists could thus benefit from thinking about such physical properties that of course fit within a structure-function paradigm.

9.3 The Influence of Lamina Composition on the Mechanical Properties of the Nucleus

Micropipette aspiration experiments have enabled the detailed study of nuclear mechanical properties by measuring the rate of deformation under pressure (Fig. 9.2a, b; Dahl et al. 2005; Pajerowski et al. 2007). By examining nuclei with different lamina compositions, it is thus possible to approximate the characteristic contributions to nuclear mechanical properties from A-type and B-type lamins (Fig. 9.2c; Shin et al. 2013; Swift et al. 2013a, b; Harada et al. 2014). The nuclear response in deformation is a combination of elastic (spring-like) and viscous (liquid-like, flowing) properties, with lamin-B's contributing primarily to the elastic response and lamin-A,C contributing viscosity. Thus the difference between a nucleus stoichiometrically dominated by A-type vs. B-type lamins might be akin to comparing a balloon filled with honey to one filled with water. The importance of A-type lamin in maintaining nuclear structural integrity and cell viability has been appreciated for many years (e.g., Broers et al. 2004; Lammerding et al. 2006), and its influence on nuclear viscosity has been more recently demonstrated in studies where nuclei are deformed during migration through microfluidic circuits (Rowat et al. 2013) or transwell pores (discussed later, Shin et al. 2013; Harada et al. 2014).

"Laminopathies" are a family of diseases that are caused by mutations in lamin-A,C (reviewed, for example, by Butin-Israeli et al. 2012; Worman 2012). These disorders include muscular dystrophies (Bonne et al. 1999), cardiomyopathies (Fatkin et al. 1999), lipodystrophies (Hegele et al. 2000; Shackleton et al. 2000;

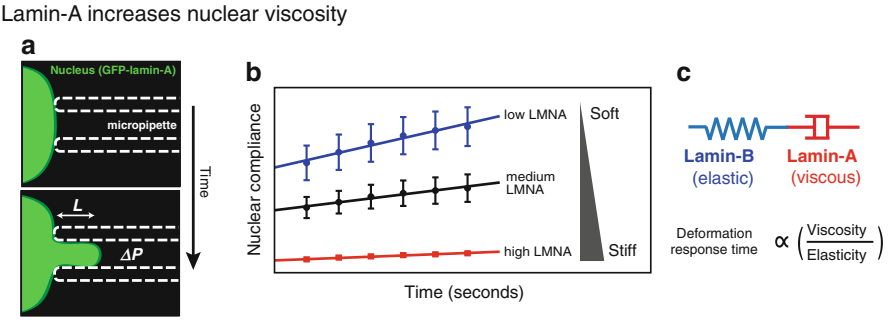

Fig. 9.2 The mechanical role of lamin in the nucleus. (**a**) Deformations applied by micropipette aspiration were used to quantify nuclear compliance (effectively a measure of "softness"; the inverse of stiffness) in a range of nuclei with altered lamina compositions (for example, by overexpressing a GFP-lamin-A fusion construct). Compliance can be calculated over a range of deformation timescales as a function of the micropipette diameter, the extent of deformation (L), and the applied pressure (ΔP). (**b**) When a constant deforming pressure was delivered by micropipette over timescales on the order of seconds, nuclei with low LMNA were found to be more compliant than those with high LMNA. (**c**) The mechanical properties of the lamina can be considered as a combination of elastic (spring-like) and viscous (flowing) properties, which together define the "deformation response time," the timescale over which the nuclear shape deforms under force. Nuclei with greater quantities of A-type lamins relative to B-type lamins were found to deform more slowly under stress (Swift et al. 2013a, b)

Speckman et al. 2000), and premature aging ("progeria," Merideth et al. 2008). Indeed, one of the confounding aspects of lamin-related disease is how such a widely expressed protein can cause tissue-specific symptoms. Whilst much work remains to be done to resolve this question, it is broadly consistent that laminopathies cause defects in tissues where lamin-A,C is the dominant lamin in the nucleus, i.e., bone, heart, muscle, and fat (although there are exceptions: Charcot-Marie-Tooth disorder affects the nervous system, De Sandre-Giovannoli et al. 2002). Mouse models of lamin-A,C knockout have defects in muscle and connective tissue and typically die several weeks after birth from heart failure (Sullivan et al. 1999; Kubben et al. 2011; Jahn et al. 2012). Despite the apparently constitutive expression of B-type lamins in tissue, mouse models with lamin-B1 and -B2 ablation progress through embryogenesis with eventual death due to defects in brain development (Coffinier et al. 2011; Kim et al. 2011).

9.4 Mechanisms of Lamin Regulation

Earlier discussion has posited that lamins are closely regulated to match the mechanical properties of the nucleus with the physical demands of tissue. In addition to being set by the epigenetic programming required to make a given tissue or organ, it is also important that protein levels vary in response to feedback from their surroundings. Even within bulk tissues, mechanical loading can cause inhomogeneous

straining (for example, in human articular cartilage, meniscus, and ligaments, Chan and Neu 2012), making it beneficial to have mechanisms of lamin regulation at the local, individual cell level.

The many mechanisms by which the level of lamin-A,C can be regulated are summarized in Fig. 9.3a. We showed that the transcript and protein levels of lamin-A,C are highly correlated in tissue (Swift et al. 2013a, b), suggestive of a tight regulatory feedback. A recent study of the proteome and transcriptome in mouse fibroblasts suggested that there are around ten million copies of lamin-A,C protein per cell—accounting for about 0.7 % of cellular protein mass—and around 200 copies of the *LMNA* transcript (Schwanhausser et al. 2011), which seems similar to single cell measurements (Dingal et al. 2015). Half-life in the cell on rigid plastic dishes was found to be around 4 days for the protein and about 20 h for the mRNA, both slightly higher than the cellular average for all proteins and genes (Schwanhausser et al. 2011). Measurements made on proteins in a human lung cancer cell line showed the half-life of lamin-A,C to be around 12 h, roughly in the middle of the span of protein-half lives recorded in the study (Eden et al. 2011). DNA methylation is known to be an epigenetic mechanism by which gene activity can be regulated, but was discounted as the foremost means of controlling *LMNA* levels: no consistent changes were observed in the methylation of the *LMNA* promoter in a range of cell lines known to express different levels of lamin-A,C protein (Swift et al. 2013 b), or in tissues from patients with laminopathic disorders (Cortese et al. 2007). *LMNA* transcription has been reported to be controlled by transcription factors of the retinoic acid (RA) receptor (RAR and RXR family proteins, Olins et al. 2001; Okumura et al. 2004a, b; Shin et al. 2013; Swift et al. 2013a, b), with the resulting mRNA alternatively spliced to give the lamin-A and truncated -C forms. Soft tissue generally favors the lamin-C spliceoform (Swift et al. 2013a, b), and in brain the micro interfering-RNA *MIR-9* specifically targets and deactivates the mRNA of the lamin-A spliceoform (Jung et al. 2012, 2013).

9.5 Stress-Responsive Regulation of Lamin: "Use It or Lose It"

To better understand how lamin proteins are actively regulated in response to stress, mesenchymal stem cells (MSCs) were cultured on collagen-1 coated polyacrylamide hydrogels with stiffnesses that set to mimic the ECM of either brain (0.3 kPa) or pre-calcified bone (40 kPa) (Buxboim et al. 2010; Swift et al. 2013b). Images of the cultured MSCs showed that the nuclear envelopes of cells on soft matrix are wrinkled and relaxed, whereas, on stiff matrix, the nuclei are flattened by stress fibers and appear taut and smooth (Fig. 9.3b—left). Accompanying proteomic analyses revealed that, on stiff matrix, the conformation of lamin-A,C protein is maintained, the total quantity is upregulated, and the extent of phosphorylation at four sites is decreased. Phosphorylation is recognized as a key mechanism for modulating the solubility, conformation, and organization of IF proteins (Omary et al. 2006),

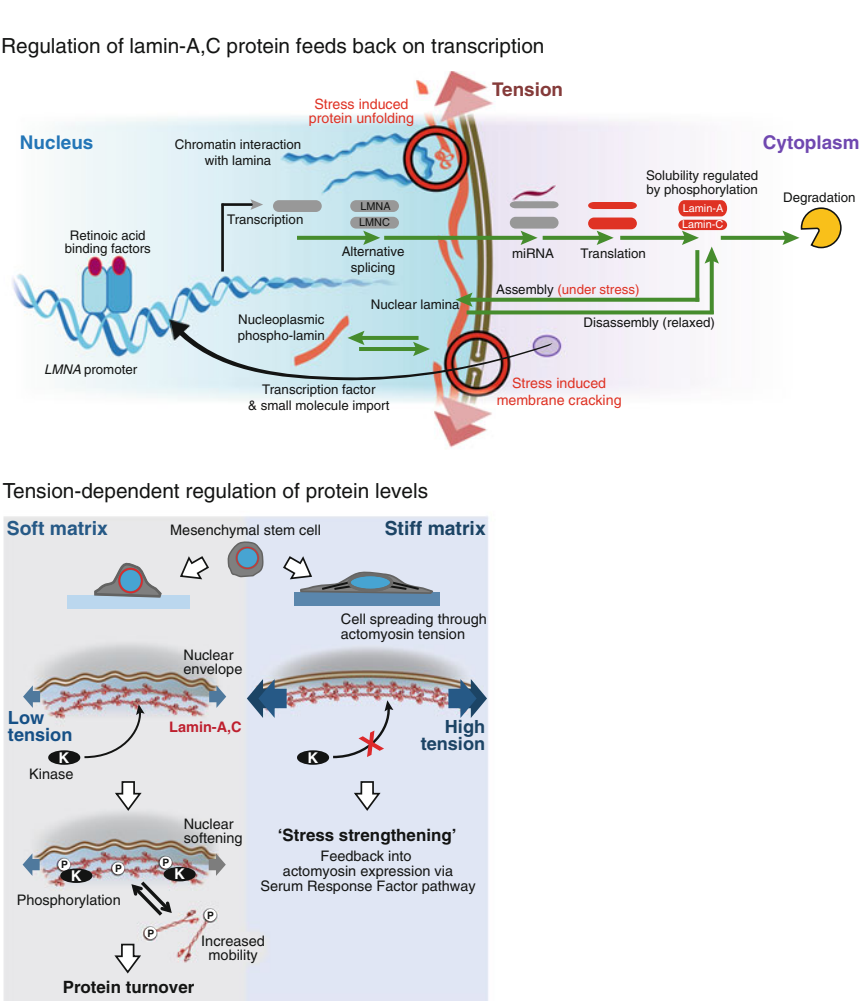

Fig. 9.3 Protein regulation as a function of stress. (**a**) Schematic showing the factors that can regulate the levels of lamin-A,C in the cell: *LMNA* transcription is promoted by retinoic acid binding factors (Olins et al. 2001; Okumura et al. 2004a, b). The transcript is alternatively spliced to give rise to lamin-A and -C forms. In some tissues such as brain the -A form is suppressed through micro interfering RNA (Jung et al. 2012). Mature lamin-A (following posttranslational processing) and lamin-C assemble into the nuclear lamina, although some protein remains mobile in the nucleoplasm (Shimi et al. 2008). Phosphorylation leads to increased solubility, and may precede enzymatic protein turnover. Further stress-dependent pathways have been reported: stress on the nucleus causes unfolding of the Ig-domain of lamin-A,C and phosphorylation is suppressed under tension (Swift et al. 2013a, b). Laminopathic nuclei have been shown to have transient membrane defects that allow ingress of transcription factors (De Vos et al. 2011). (**b**) The nuclei of MSCs cultured on soft substrate were wrinkled, whereas those in cells on stiff substrate had a smooth, stretched appearance suggestive of greater tension. We have shown that lamin-A,C is less phosphorylated under tension (Swift et al. 2013a, b). By concentrating on one of the matrix-stiffness-regulated phosphorylation sites, we confirmed that lamin-A,C is rapidly phosphorylated with reduced cytoskeleton tension and phosphorylation leads to nuclear softening and lamin-A,C turnover (Buxboim et al. 2014)

and indeed lamins are highly phosphorylated during normal mitosis, driving disassembly of the lamina in preparation for chromosomal separation (Gerace and Blobel 1980; Heald and McKeon 1990). Thus the response we observe from matrix-induced stress is the converse of this process, with decreased phosphorylation acting to decrease lamin-A,C solubility and thereby strengthening the lamina. On soft matrix, lamin-A,C is more extensively phosphorylated, more mobile, and so, more susceptible to turnover (Buxboim et al. 2014). These observations hence point to a "use it or lose it" dynamic, whereby inessential lamin-A,C is eventually degraded. Lamin-A,C level has been reported to drive the translocation of the lamin-promoting transcription factor retinoic acid receptor gamma (RARG) to the nucleus, pointing to a feedback mechanism by which lamin protein levels promote their own transcription (see gene circuit in Swift et al. 2013a, b).

9.6 Mechanotransduction to the Nucleus: Downstream of Matrix and Lamin

Lamin is a key component in a system of protein linkages that allow the transmission of signals from a cell's surroundings into the transcriptional machinery of its nucleus (Fig. 9.1a and discussed in recent reviews, e.g., Simon and Wilson 2011; Gundersen and Worman 2013; Rothballer and Kutay 2013; Sosa et al. 2013). Cell–cell interactions link to the cytoskeleton through tight and adherens junctions that tether to actin, and desmosome complexes that interact with cytoplasmic IFs such as keratin (Jamora and Fuchs 2002). Cell–matrix interactions are mediated by integrins and focal adhesion complexes that bind to cytoplasmic actin (Puklin-Faucher and Sheetz 2009; Watt and Huck 2013). The appropriately named LINC complex ("linker of nucleo- and cytoskeleton") acts as an intermediary between cytoplasmic and nuclear structural proteins: F-actin binds to the nuclear envelope components nesprins -1 and -2, and IFs bind to the desmosome protein plectin, which in turn binds nesprin-3. Nesprins can also interact with kinesin and dynein complexes to tether to the microtubule network; Nesprins bind the SUN domain-containing family of inner nuclear membrane proteins and these in turn bind to the lamina on the inside of the nuclear envelope. Current problems for progress on understanding the roles of Nesprins are that there are few if any good antibodies to Nesprins and there are many spliceforms of Nesprins.

Lamin interactions within the nucleus are highly promiscuous (Wilson and Berk 2010; Wilson and Foisner 2010); as emphasized by Wilson and Berk in their review: "almost all characterized [inner nuclear membrane] proteins bind to A- or B-type lamins (or both) directly." These interactions include binding to structural proteins, like actin (Simon et al. 2010), and a range of proteins that bind to the nuclear membrane, including emerin, barrier-to-autointegration factor (BAF, de Oca et al. 2009), lamina-associated polypeptide 2 (LAP2), and lamin-B receptor (LBR, Solovei et al. 2013). Of these, emerin has attracted considerable recent interest for its roles in mediating changes in the stiffness of isolated nuclei in response to tension applied

to nesprin-1 (Guilluy et al. 2014), and in mechanosensing by affecting the translocation of transcription factor MKL1 (Ho et al. 2013). Furthermore, some transcription factors such as Oct-1 interact with the lamina directly (Malhas et al. 2009). Many lamin-binding proteins also interact with chromatin, particularly in its silenced heterochromatin form (Wagner and Krohne 2007), and indeed the lamins have been shown to bind DNA directly (Shoeman and Traub 1990; Luderus et al. 1992; Stierle et al. 2003). This chain of interactions thus completes a continuous physical linkage through which deformations can be transmitted from the cell exterior to chromatin (Maniotis et al. 1997). What is missing from this picture, however, is how blunt inputs—forces and perturbations acting without microscopic coherence—can be converted from mechanical to biochemical signals to activate individual genes at precise spatial locations within the nucleus. Perhaps specificity can be delivered through changes in binding, local concentration, conformation, and modification of cofactors or transcription factors.

As described above, mechanical cues from outside the cell alter protein conformations, protein modifications, and protein levels—all of which can broadly affect cell morphology and function. It is therefore of particular interest to understand the multiplicity of mechanisms that likely underlie how external factors induce stem cell lineage, with far-reaching implications for therapeutics and regenerative medicine. Populations of MSCs can be expanded in culture in a relatively naïve undifferentiated state, but they can certainly differentiate into multiple mesenchymal lineages, including fat, cartilage, muscle, and bone, dependent on external cues, such as the presence of nutrients, growth factors and cytokines, cell density, spatial constraints and mechanical forces (Pittenger et al. 1999). Cell shape influences cell fate through modulation of the activity of the small GTPase RhoA, with round cells favoring adipogenesis and well-spread cells favoring osteogenic lineage (McBeath et al. 2004). RhoA drives commitment to lineage in conjunction with its effector Rho-associated protein kinase (ROCK) through its regulation of nonmuscle myosin-II that controls cytoskeletal tension.

Although the focus of this review on the nucleus limits a deeper discussion of nonmuscle myosin-II, at least two points should be made. Knockout mice that completely lack nonmuscle myosin-IIA (MYH9) die at such an early embryonic stage that they exhibit little to no differentiation: no heart and no vasculature (Conti et al. 2004). This myosin-II isoform tends to be the dominant and early form of nonmuscle myosin-II isoforms in many tissues. Nonetheless, heterozygous mutations in human MYH9 are common and exhibit weak dominant negative effects on the wild-type protein from the normal allele (Spinler et al. 2015), which strongly suggests that even less than half of nonmuscle myosin-IIA is sufficient for near-normal differentiation in most tissues.

Cell fate can be influenced by matrix stiffness (Engler et al. 2006), and we have recently shown that this effect can be modulated by the nuclear lamina (Fig. 9.4a; Swift et al. 2013a, b). MSCs cultured on soft hydrogel substrates favor adipogenesis, but the extent of adipogenesis, as determined by oil red staining of lipid droplets, is double or more when combined with lamin-A,C knockdown. Likewise, stiff matrix induction of osteogenesis is greatly increased by lamin-A overexpression. Stiff

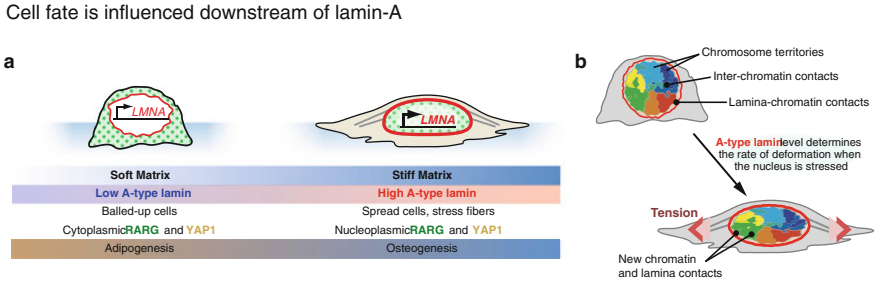

Fig. 9.4 Decisions of cell fate downstream of lamin-A,C regulation. (**a**) MSCs cultured on soft and stiff substrates take on differing phenotypes and favor alternate cell fates (Engler et al. 2006). On soft substrate, MSCs exhibit small nuclear and cellular spread areas and the nuclear lamina is thinned by a stress-sensitive phosphorylation feedback mechanism (Fig. 9.3b—left; Swift et al. 2013a, b). The transcription factors RARG and YAP1 (YAP1, Dupont et al. 2011) remain in the cytoplasm, and adipogenic cell fate is preferred. On stiff substrate, cells spread extensively with nuclei that are pinned down by well developed stress fibers. Lamin-A,C is less phosphorylated under strain, thus strengthening the lamina; RARG also translocates to the nucleus, increasing *LMNA* transcription. Activity of the transcription factor SRF (downstream of lamin-A,C) increases expression of cytoskeletal components (Ho et al. 2013; Swift et al. 2013b). Under these conditions, YAP1 translocates to the nucleus and cells favor osteogenesis. On both soft and stiff substrates, the effects of matrix elasticity and lamin level cooperate to enhance differentiation: lamin-A,C knockdown on soft matrix leads to more adipogenesis; lamin-A overexpression on stiff matrix leads to more osteogenesis. (**b**) Transcriptional activity is believed to be regulated by conserved interchromatin contacts that give rise to the spatial ordering of chromosomes (chromosome territories shown here in different colors, Cremer and Cremer 2001). Lamin-A,C can interact with DNA directly ("lamina-chromatin contacts") or through protein intermediaries (Simon and Wilson 2011), but could have additional regulatory roles by mechanically determining the extent and rate that the nucleus deforms under tension, a process that could lead to the formation of altered interchromatin and lamina-chromatin contacts

matrix has been found to drive the nuclear translocation of the transcription factors RARG (Fig. 9.4a; Swift et al. 2013a, b) and yes-associated protein 1 (YAP1) (Dupont et al. 2011). RARG directly regulates lamin-A,C as part of differentiation and regulation of the serum response factor (SRF) pathway that amplifies levels of cytoskeletal components such as nonmuscle myosin-IIA (Fig. 9.4a; Swift et al. 2013a, b). Findings with MSCs on stiff matrix indeed are consistent with greater SRF activity in epithelial cells (Connelly et al. 2010; Ho et al. 2013). NKX2.5 is yet another transcription factor that is matrix elasticity sensitive, but it accumulates in the nucleus of MSCs on soft matrix and represses expression of at least one tension stabilizing protein, smooth muscle actin (SMA) (Dingal et al. 2015). Nuclear translocation of transcriptional regulators in response to matrix mechanics is thus an emerging theme in mechanosensing. It has also been shown to occur upon transfer of ions and changes in osmotic pressure (Finan et al. 2009; Irianto et al. 2013; Kalinowski et al. 2013). Such translocation could be driven by a change in concentration of protein-binding sites (e.g., on lamin-A,C or emerin, Ho et al. 2013; Swift et al. 2013a, b) or conceivably by protein modifications (e.g., YAP1 nuclear localization can be mediated by phosphorylation, Murphy et al. 2014). Besides

conventional transport through nuclear pores via nuclear localization sequences (NLS) (Dingal et al. 2015), transient breakdown of the nuclear envelope in cells with defects in the lamina, perhaps as a consequence of a reduced robustness to mechanical stress, has been shown to affect nuclear localization of transcription factors such as RelA (De Vos et al. 2011).

The ability of lamins and/or its binding partners to tether to DNA has lead to interest in its role in chromatin organization and regulation (Guelen et al. 2008; Kim et al. 2011; Zullo et al. 2012; Kind et al. 2013; Lund et al. 2013; Meuleman et al. 2013). Lamina-associated domains (LADs) located at the nuclear periphery have been thought to associate with low gene expression levels whereas actively transcribed euchromatin is usually found in the nuclear interior. This might contribute to "chromosome territories" (Cremer and Cremer 2001; Iyer et al. 2012) and to transcriptional hotspots within specific locations (Fraser and Bickmore 2007). However, it has been recently determined that nuclear lamins are not required for LAD organization in embryonic stem cells (Amendola and van Steensel 2015). Chromatin and DNA are also generally considered to make negligible contributions to overall nuclear mechanics (Pajerowski et al. 2007; Guilluy et al. 2014) unless the nucleus is condensed (Pajerowski et al. 2007), although particular cases are emerging—for example, in ESCs passing though a metastable transitional state before differentiation—where the condensation state of chromatin can become mechanically significant (Pagliara et al. 2014). It is not yet fully understood which proteins could give rise to mechanically responsive, locally defined structures and organization within the nucleus, nor how a protein as ubiquitously expressed as lamin could play a part in such specificity. Knowledge in this field will continue to improve as new experiments and models emerge to study protein-mediated changes in chromatin organization in response to perturbation (Shivashankar 2011; Talwar et al. 2013). However, based on current work, we have hypothesized that the effect of lamins on nuclear mechanics could determine the sensitivity and timescale of nuclear reorganization in response to stress (Fig. 9.4b; Swift et al. 2013a, b).

9.7 Cell Migration Is Slowed by the Nuclear Stiffness Needed to Protect Chromatin

As the nucleus is generally the largest and stiffest organelle, it can be a limiting factor in the migration of a cell through the 3D matrix. This means that the mechanical properties of the nucleus can have regulatory roles in processes, such as development, wound healing, hematopoiesis, cancer metastasis, and others (Fig. 9.5a—left). Studies of migration through narrow pores that mimic those in tumor tissue and require the deformation of the nucleus demonstrated a dependence on lamina composition; migration was limited when lamin-A was overexpressed, but promoted by a ~50 % knockdown of the protein (Harada et al. 2014). However, a deeper knockdown to <10 % was found to cause apoptosis in migration through small pores,

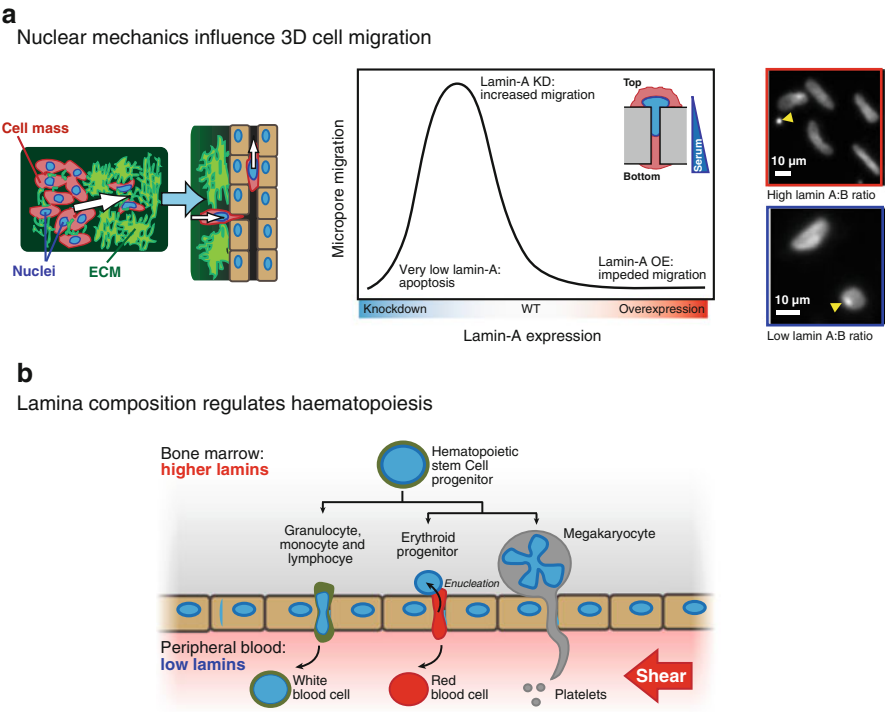

Fig. 9.5 The influence of the mechanical properties of the nucleus on cell migration. ((**a**)—**left**) As the largest and stiffest organelle in the cell, the nucleus can act as an "anchor" and prevents cell movement through the matrix or into surrounding vasculature. ((**a**)—**center**) As a model of migration through matrix, cells are induced to pass through 3 μm pores, a diameter sufficiently small to require deformation of the nucleus (*inset*). Lamin-A overexpression inhibits migration, whereas knockdown increases migration, up to a point at which significant apoptosis is observed. Thus extremely low or high lamin-A,C levels are unfavorable for cell migration, an observation with potential impact on understanding of processes such as cell migration during development and cancer metastasis. ((**a**)—**right**) Lamin-A rich nuclei (top image) showed persistence of a sausage-like morphology upon emergence from the pores (*yellow arrow*), while lamin-B rich nuclei (bottom image) rapidly recovered their shape (Fig. 9.5a adapted from ©Harada et al. 2014. Originally published in The Journal of Cell Biology. doi: 10.1083/jcb.201308029). (**b**) Effect of lamina composition on nuclear deformability during hematopoiesis. Stem cells that are retained in the marrow niche have higher lamin levels than differentiated blood lineages (Shin et al. 2013). A downregulation of nuclear cytoskeletal components in granulocytes, for example, ostensibly makes the cells better suited for passage through narrow blood vessels, but the lack of nuclear stability may contribute to their relatively short circulation times (Olins et al. 2009)

underscoring the importance of lamin in providing physical protection to the nucleus (Fig. 9.5a—center). Consistent with earlier observations that lamins -A and -B, respectively, contribute primarily viscous and elastic mechanical properties to nuclei (Fig. 9.2), nuclei in which high lamin-A,C levels dominated the mechanical characteristics were observed to recover slowly following deformation, maintaining an elongated morphology after emerging from the pores (Fig. 9.5a—top right

image). In contrast, nuclei with dominant levels of elastic B-type lamins rapidly returned to their more spheroid pre-migratory shapes following deformation (Fig. 9.5a—bottom right image).

Cell migration is an important part of the development process and it is possible that the elasticity imparted by lamin-B is needed to allow nuclei to recover from the deformation (typically elongation) that occurs during migration, perhaps explaining why the brain fails to develop in lamin-B knockout mice (Coffinier et al. 2011; Kim et al. 2011; Jung et al. 2013). Neutrophilic cells also have very low levels of nucleoskeletal proteins to allow their deformation as they squeeze into confined spaces (Olins et al. 2009; Rowat et al. 2013), and indeed the composition of the nuclear lamina is continuously regulated during hematopoiesis (Fig. 9.5b; Shin et al. 2013). We hypothesize that by downregulating components of the lamina, white blood cells compromise their robustness in favor of mobility, and that this contributes to the short lifetimes of many of these cells in circulation. Cancer metastasis is an equally complex process that depends on factors including matrix remodeling and nuclear deformability (Wolf et al. 2013; Harada et al. 2014). Other work has shown that myosin-II's ability to deform the nucleus can be a decisive factor in limiting glioma migration into brain tissue (Beadle et al. 2008; Ivkovic et al. 2012), but cancer cells in general show no universal lamina phenotype (reviewed in Foster et al. 2010). Although low levels of lamin-A,C have been correlated with increased reoccurrence of colon cancers (Belt et al. 2011), lamin-A,C was found to be upregulated in certain skin and ovarian cancers (Tilli et al. 2003; Hudson et al. 2007) and higher lamin-A,C expression was associated with better clinical outcomes in breast cancer (Wazir et al. 2013). Our own studies of tumor expansion in mouse flank have associated moderately lower levels of lamin-A,C with an increased invasiveness into the surrounding tissue, but a more complex dependence of the level of lamin-A,C with clinical prognosis might be explained by the tenuous balance between the effect of the lamina on nuclear deformability compared with that on cell survival.

9.8 Lamins in Cancer

Many studies have shown that lamin levels change in cancer of many organ types when compared to normal tissue (Table 9.1). Direct mechanistic links between lamins and cancer progression remain mysterious nonetheless. In cancer progression, a proliferation-competent cell acquires a cancer phenotype by either epigenetic changes (DNA methylation and histone modifications, Berdasco and Esteller 2010) or direct genomic changes (mutational, Salk et al. 2010) that lead to activation of oncogenes or inactivation of tumor suppressor genes (for review Hanahan and Weinberg 2011). Many in vitro studies have suggested a role for lamin-A in DNA damage response (Musich and Zou 2009; Mahen et al. 2013; Singh et al. 2013), but mice and humans with lamin-A deficiencies and defects are not reported to have an increased risk of cancer. Nonetheless, deep knockdown of lamin-A increases

Table 9.1 Lamins in cancer

Type of cancer	Lamin-A,C	Lamin-B
Lung cancer	↓	
Breast cancer	↓	↑
Colon cancer	↓	↓
Colorectal cancer	↑	↑
Colonic and gastric adenocarcinomas	↓	
Primary gastric carcinoma	↓	
Basal cell skin carcinoma	↓	
Skin cancer	↑	
Leukemia	↓	
Ovarian serous cancer	↑	
Ovarian cancer	↓	↑
Prostate cancer	↓	↑
Liver cancer		↑
Pancreatic cancer		↑

apoptosis after constrained migration through small matrix-like pores, consistent with increased DNA damage (Harada et al. 2014). The same study also showed migration—induced damage of the nuclear lamina, and nuclear ruptures have been observed in cancer cells (Vargas et al. 2012). These findings collectively suggest a protective role of lamins as an "armor" for guarding the genome. Further work is required to drill into the mechanistic link between lamins and cancer, which may lead to new treatments or at least a clearer basis for lamins as bio-marker in cancer progression.

9.9 Conclusions and Prospects

We have sought to outline the importance of nuclear mechanics in the context of tissue function, considering how it reflects the protective properties of the lamina, influences cell fate, and also regulates cell migration. In understanding that one of the key functions of the lamins is to ensure that the mechanical properties of the cell meets the demands of a tissue—either directly or by driving broader changes with regard to cell fate—lamins stress response factors. The response to cellular stress is classically thought in terms of how cells mitigate "heat-shock" that otherwise result in high levels of unfolded proteins (Hartl et al. 2011), but mechanical stress might also cause chromatin unfolding. Nonetheless, we are still a long way from understanding cellular protection mechanisms and how stress response pathways affect the regulation of structural features within the cell—motivating more work in nuclear biophysics.

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