Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction

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Mutations in the lamin A/C gene (LMNA) cause a variety of human diseases including Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy, and Hutchinson-Gilford progeria syndrome. The tissue-specific effects of lamin mutations are unclear, in part because the function of lamin A/C is incompletely defined, but the many muscle-specific phenotypes suggest that defective lamin A/C could increase cellular mechanical sensitivity. To investigate the role of lamin A/C in mechanotransduction, we subjected lamin A/C–deficient mouse embryo fibroblasts to mechanical strain and measured nuclear mechanical properties and strain-induced signaling. We found that Lmna−/− cells have increased nuclear deformation, defective mechanotransduction, and impaired viability under mechanical strain. NF-κB–regulated transcription in response to mechanical or cytokine stimulation was attenuated in Lmna−/− cells despite increased transcription factor binding. Lamin A/C deficiency is thus associated with both defective nuclear mechanics and impaired mechanically activated gene transcription. These findings suggest that the tissue-specific effects of lamin A/C mutations observed in the laminopathies may arise from varying degrees of impaired nuclear mechanics and transcriptional activation.


Introduction
Lamins are structural components of the nuclear lamina, a protein network underlying the inner nuclear membrane that determines nuclear shape and size (1). In addition, lamins play an important role in organizing nuclear pore complexes (2) and recruiting other proteins such as emerin to the nuclear envelope (3, 4).

Two types of lamins are found in mammalian cells: A-type lamins (lamin A, C, A∆10, and C2) are encoded by a single gene (Lmna) and are developmentally regulated and expressed in differentiated cells. B-type lamins (B1 and B2/B3) are encoded by two distinct genes and are constitutively expressed in all cells (1, 5–10). Mutations in the gene encoding A-type lamins and their binding partners have been associated with a variety of human diseases, including Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy, Dunningan-type familial partial lipodystrophy, and Hutchinson-Gilford progeria syndrome (11–17).

The molecular mechanisms underlying the varied phenotypes are unknown, and two alternative hypotheses have been proposed to explain the tissue-specific effects observed in laminopathies. The “structural hypothesis” suggests that lamin mutations lead to increased nuclear fragility and eventual nuclear disruption in the mechanically stressed tissue, while the “gene regulation hypothesis” proposes a tissue-specific role of lamins in DNA transcription. Lmna−/− mice are indistinguishable from their littermates at birth but develop severe muscle wasting and contractures similar to Emery-Dreifuss muscular dystrophy by 3–4 weeks and die by 8 weeks (4). Cells derived from Lmna−/− mice have misshapen nuclei and obvious ultrastructural damage (4, 18). Distorted nuclear shape has also been demonstrated in fibroblasts from lipodystrophic patients with heterozygous R482Q/W mutations in the lamin A/C gene and in cells from Caenorhabditis elegans with reduced lamin levels (1, 19). Here we show that nuclear mechanics in cells from Lmna−/− mice are defective, with Lmna−/− nuclei displaying increased deformation and fragility under strain. In addition, we demonstrate that transcriptional activation in response to mechanical stimuli is attenuated in Lmna−/− cells, impairing viability of mechanically strained cells. These data suggest that the structural and gene regulation hypotheses of the laminopathies are in fact closely related, and different mutations may cause specific phenotypes by differentially affecting these processes.
Methods

Cells. Lmna+/+ and Lmna−/− mouse embryo fibroblasts were maintained in DMEM (Invitrogen Corp., Carlsbad, California, USA) containing 10% FCS (HyClone Laboratories, Logan, Utah, USA) and penicillin/streptomycin (Invitrogen Corp.).

Nuclear strain experiments. Cells were plated at 900 cells/cm² on fibronectin-coated silicone membranes in DMEM supplemented with 10% FCS followed by serum starvation for 48 hours in DMEM containing insulin, transferrin, and selenium (ITS) supplement (Sigma-Aldrich, St. Louis, Missouri, USA). Preceding the strain experiments, cells were incubated with Hoechst 33342 nuclear stain (1 μg/ml; Molecular Probes Inc., Eugene, Oregon, USA) or for 30 minutes. To minimize nuclear effects, cells were incubated with fibronectin-coated paramagnetic beads (Bangs Laboratories Inc., Fishers, Indiana, USA) were incubated together with the magnetic bead contact radius of 2 μm.

Magnetic bead microrheology. Cells were plated on 35-mm polystyrene dishes (Corning-Costar Corp., Corning, New York, USA). The following day, cells were incubated with fibronectin-coated paramagnetic beads (Dynal Biotech Inc., Lake Success, New York, USA) for 30 minutes. To minimize nuclear effects, only beads attached more than 5 μm from the nuclei were selected for analysis. A sinusoidal force (amplitude 0.6 nanonewtons [nN], frequency 1 Hz, offset 0.6 nN) was applied through a magnetic trap, and bead displacement was monitored using a digital camera (Roper Scientific Inc., San Diego, California, USA). Displacement amplitudes were computed using custom-written MATLAB (The MathWorks Inc., Natick, Massachusetts, USA) algorithms. In separate experiments, smaller (2 μm) fibronectin-coated polystyrene beads (Bangs Laboratories Inc., Fishers, Indiana, USA) were incubated together with the magnetic beads for 1 hour to adhere to the cell surface. Cells containing single magnetic beads and several polystyrene beads were subjected to a brief force pulse (2.5 nN for 3 seconds). Using custom-written MATLAB algorithms, maximal induced magnetic and polystyrene bead displacements were computed and expressed in cylindrical coordinates (r, θ) with the magnetic bead at the origin and θ = 0 for the force direction. The induced strain field can be described by an analytical cell mechanics model proposed by Bausch et al. (20) expressing the radial component ur of the induced bead displacement as a function of the applied force F, cell stiffness μ, the characteristic cut of radius κ−1, the distance from the magnetic bead center r, and the polar angle θ:

Equation 1

where K₀ and K₁ are modified Bessel functions of the second kind (order 0 and 1, respectively) and using κ₁ = [(1 − σ)/2]1/2κ. The parameters μ, and κ were obtained by fitting equation 1 to the bead displacement data using the GraphPad Prism 4.0 robust curve fit function (GraphPad Software, San Diego, California, USA) and assuming σ = 0.5 for incompressible media and a magnetic bead contact radius of 2 μm.

The magnetic trap calibration was performed as described previously (21). In brief, magnetic beads suspended in viscous solution were monitored while being attracted to the magnetic trap operated at various currents. The applied force as a function of current and distance from the magnetic trap was then computed based on Stokes’ law.

Microinjection. Cells were plated on fibronectin-coated glass dishes (WillCo Wells BV, Amsterdam, The Netherlands) or silicone dishes and incubated overnight. Microinjections were performed using an Eppendorf microinjector with Eppendorf Femtotips (Eppendorf AG, Hamburg, Germany). In each dish, 20–50 cells were injected with Texas Red–labeled 70-kDa dextran (Molecular Probes Inc.) dissolved at 10 mg/ml in PBS (Invitrogen Corp.), either into the cytoplasm (injection pressure 500 hectopascals [hPa], injection time 0.6 seconds) or into the nucleus (injection pressure 10, 100, 500, and 1,500 hPa, injection time 0.6 seconds). Following microinjection, cells were washed in HBSS (Invitrogen Corp.), and intracellular localization of dextran–Texas Red was recorded under a fluorescent microscope. Selected silicone membranes were subjected to constant biaxial strain (about 32% for 30 minutes) or cyclic biaxial strain (10% at 1 Hz for 24 hours), and localization of Texas Red–labeled dextran in strained and control cells was analyzed on a fluorescence microscope (Olympus America Inc.).

Strain experiments. Strain stimulation was carried out as previously described (22). In brief, cells were plated on fibronectin-coated silicone membranes (2,500–3,300 cells/cm²). After 72 hours of serum starvation, cells were subjected to biaxial cyclic strain (4% or 10% at 1 Hz). For chemical stimulation, cells were incubated with IL-1β (25 ng/ml; R&D Systems Inc., Minneapolis, Minnesota, USA) or PMA (200 ng/ml, Sigma-Aldrich) in DMEM plus ITS.

DsRed/peroxiredoxin-2 localization. Cells were plated on fibronectin-coated glass dishes or fibronectin-coated silicone membranes. Following overnight incubation, cells were transfected with a CMV promoter–driven DsRed/peroxiredoxin-2 fusion construct (CLONTECH Inc., Palo Alto, California, USA) using FuGENE 6 (F. Hoffman–La Roche Ltd., Basel, Switzerland) and incubated for 24 hours. Selected silicone membranes were subjected to constant (about 19% for 60 minutes) or cyclic biaxial strain (10% at 1 Hz for 3 hours), and
localization of DsRed-labeled peroxiredoxin-2 in stained and control cells was analyzed on a fluorescence microscope (Olympus America Inc.).

Flow cytometry. For cell viability assays, propidium iodide (2 μg/ml, Sigma-Aldrich) was added to the dishes after 24 hours of strain application. Cells were collected and analyzed using a Cytomics FC 500 flow cytometer (Beckman Coulter Inc., Fullerton, California, USA), counting 10,000–30,000 events in each group. Thresholds for propidium iodide incorporation were determined based on negative (no propidium iodide staining) and positive (cells permeabilized by 50% ethanol) controls. Apoptotic and necrotic cell fractions were measured in similar experiments using the Vybrant Apoptosis Assay Kit no. 3 (Molecular Probes Inc.).

Northern and Western analyses. Expression of iex-1 and egr-1 mRNA was assessed by Northern analysis as described previously (23). Protein expression was analyzed by Western analysis of nuclear and cytoplasmic cell fractions using antibodies against NF-κB p65 (antibody does not recognize p50 or p105), IkBα (both from Santa Cruz Biotechnology Inc., Santa Cruz, California, USA), and actin (Sigma-Aldrich). Additional immunoblotting was performed on whole cell lysates using specific antibodies against total ERK1/2 (Santa Cruz Biotechnology Inc.) and phospho-p44/p42 MAP kinase (Cell Signaling Technology, Beverly, Massachusetts, USA), specific bands were visualized by enhanced chemiluminescence (PerkinElmer Inc., Boston, Massachusetts, USA).

Luciferase experiments. Cells were transfected with plasmids for NF-κB–controlled luciferase expression and SV40-regulated β-gal (Promega Corp., Madison, Wisconsin, USA) using FuGENE 6 (F. Hoffmann-La Roche Ltd.). Following transfection, cells were serum starved in DMEM plus ITS medium for 48 hours, followed by overnight stimulation with PMA (200 ng/ml) or IL-1β (25 ng/ml). Luciferase assays were quantified in a Victor2 Multilabel Counter (Perkin Elmer Inc.). Results were normalized for β-gal activity and expressed as percent WT control.

Immunohistochemistry. Cells were plated on untreated or fibronectin-coated chamber slides and serum-starved for 24–72 hours followed by stimulation with IL-1β. Cells were fixed in 4% paraformaldehyde or methanol, washed in PBS, and permeabilized with 0.1% Triton X-100. After blocking, cells were incubated overnight with primary rabbit antibody anti–NF-κB (p65; Santa Cruz Biotechnology Inc.) at 4°C or Alexa Fluor 568 phalloidin (A-12380; Molecular Probes Inc.) for 1 hour at 25°C, followed by 1 hour of incubation with secondary FITC- or TRITC-conjugated antibodies.

Cellular protein fractions and electrophoretic mobility shift assay. Nuclear extracts were prepared as described previously (24) with the following modifications. Cells were washed with ice-cold PBS and lysed in buffer A, which consisted of 0.1% Triton X-100, 10 mM EDTA, 10 mM EGTA, 10 mM KCl, 10 mM HEPES, 1 mM DTT, 0.5 mM PMSF, and protease inhibitor cocktail (P-8340; Sigma-Aldrich). After centrifugation at 1,200 g for 10 minutes, the supernatant was stored as the cytoplasmic cell fraction, while the nuclear pellet was washed once in PBS, resuspended in buffer C (1 mM EDTA, 1 mM EGTA, 0.4 M NaCl, 20 mM HEPES, 5 mM MgCl₂, 25% glycerol, 1 mM DTT, 0.5 mM PMSF, and protease inhibitor cocktail), and incubated at 4°C for 10 minutes. The nuclear extract was centrifuged for 10 minutes at 4°C at 12,000 g, and the supernatant was used for Western analysis and electrophoretic mobility shift assay. NF-κB–specific oligonucleotides (Promega Corp.) were end-labeled using T4 polynucleotide kinase and [γ-32P]ATP (DuPont NEN Research Products, Boston, Massachusetts, USA). Nuclear extracts were preincubated for 10 minutes in binding buffer followed by 20 minutes of incubation at room temperature with labeled oligonucleotide. Samples were separated on a 4% native polyacrylamide gel. For competition studies, an excess (50 times) of unlabeled oligonucleotide was added.

Statistical analysis. All experiments were performed at least three independent times. Data are expressed as mean ± SEM. Statistical analysis was performed using
PRISM 4.0 and INSTAT software (GraphPad Software Inc., San Diego, California, USA). The data were analyzed by unpaired t test (allowing different SDs), one-way ANOVA, or the Mann-Whitney test in case of non-parametric distribution. A two-tailed P value below 0.05 was considered significant.

**Results**

**Nuclear mechanics.** To explore the role of lamin A/C in nuclear mechanics, mouse embryo fibroblasts derived from Lmna<sup>−/−</sup> and Lmna<sup>+/+</sup> mice were cultured on transparent membranes and subjected to stepwise increasing biaxial strain (first step: 10.1% ± 0.18%; second step: 18.2% ± 0.07%). The cytoskeleton, attached to the membrane through integrin receptors, is exposed to the same biaxial strain as the membrane, while the stiffer nucleus exhibits only small deformations in WT cells (25). The induced nuclear deformations were calculated by tracking the fluorescently labeled nucleus (Figure 1, a and b) and normalized to membrane strain to compensate for the small variation in applied membrane strain. For each cell type, nuclear deformation increased approximately linearly with applied membrane strain (Figure 1c), but Lmna<sup>−/−</sup> nuclei showed significantly larger deformations than did WT cells (Figure 1, c and d). Fitting a linear regression to the nuclear deformation data revealed a significantly larger slope for the Lmna<sup>−/−</sup> nuclei, and the maximal normalized nuclear deformation was significantly larger for Lmna<sup>−/−</sup> cells, indicating impaired nuclear mechanics in lamin A/C–deficient nuclei.

**Cytoskeletal mechanics.** To evaluate the possibility that the observed increase in nuclear deformation was caused by more direct force transmission to the nucleus, we examined cytoskeletal organization by staining actin stress fibers with phalloidin. No apparent differences in cytoskeletal architecture were found between WT (Figure 2a) and Lmna<sup>−/−</sup> cells (Figure 2b). Magnetic bead microrheology (20) was used for quantitative evaluation of cytoskeletal stiffness. Small (4.5 μm), fibronectin-coated paramagnetic beads were attached to the cell, and the bead displacement in response to an applied magnetic force was used as an indicator of cytoskeletal stiffness (Figure 2c). The induced bead displacement amplitude was significantly increased in Lmna<sup>−/−</sup> fibroblasts (Figure 2d), indicating decreased cytoskeletal stiffness. Since magnetic bead displacement depends not only on cytoskeletal stiffness but also on the binding characteristics of the cell to the bead, we applied a second microrheology method that measures the induced displacement in polystyrene beads located on the cell surface close to the magnetic bead (Figure 2, e and f). Lamin A/C–deficient cells exhibited significantly larger induced polystyrene bead displacements.

**Figure 2**

Cytoskeletal stiffness is reduced in lamin A/C–deficient cells. (a) Phalloidin staining for actin stress fibers in WT (Lmna<sup>+/+</sup>) fibroblasts. Scale bar: 20 μm. (b) Phalloidin staining for actin stress fibers in Lmna<sup>−/−</sup> cells. Scale bar: 20 μm. (c) Magnetic bead microrheology. Representative examples of magnetic bead displacement in response to applied sinusoidal force (thin black line) for WT (thick black line) and Lmna<sup>−/−</sup> (thick gray line) fibroblasts. (d) Bead displacement amplitude in response to applied magnetic forces was significantly increased in Lmna<sup>−/−</sup> fibroblasts, indicating reduced cytoskeletal stiffness in lamin A/C–deficient cells (0.124 ± 0.024 μN vs. 0.226 ± 0.029 μN; P < 0.01, n = 60). (e) Fibroblast with magnetic (diameter 4.5 μm) and polystyrene beads (diameter 2 μm) attached to the cell membrane. Scale bar: 10 μm. (f) Graphic representation of the displacement field after a brief force pulse (2.5 nN for 3 seconds). Bead sizes and positions are drawn to scale, while bead deflections are enlarged by a factor of 10. (g and h) Distance dependence of the angle-corrected radial bead displacement component u/κcosθ as defined in equation 1. The dotted line is an optimal fit to equation 1, yielding estimates for cellular stiffness μ* and dissipation κ for WT (g) and Lmna<sup>−/−</sup> cells (h), respectively (μ*: 27.53 ± 8.458 μN/μm vs. 2.417 ± 734.7 μN/μm; P < 0.01, n = 128 [WT], 153 [Lmna<sup>−/−</sup>]; κ: 0.020 ± 0.017 μm<sup>−1</sup> vs. 0.201 ± 0.072 μm<sup>−1</sup>; P < 0.05, n = 128 [WT], 153 [Lmna<sup>−/−</sup>]). μN, micronewton.
that decreased quickly with increasing distance from the magnetic bead, whereas bead displacements in WT cells were smaller and hardly exceeded the detection limit of less than 0.1 µm (Figure 2, g and h). Based on an analytical model proposed by Bausch et al. (20), the induced polystyrene bead displacement is a function of the applied force, the position relative to the magnetic bead, and two cellular mechanics parameters μ* and κ that describe the cell stiffness and intracellular strain dissipation, respectively. By separately fitting the theoretical displacement field to the observed bead data for Lmna−/− and WT fibroblasts, one can estimate the parameters for cellular stiffness and dissipation (Figure 2, g and h), confirming that Lmna−/− cells exhibit significantly decreased cytoskeletal stiffness.

Therefore, the observed increased nuclear deformation in lamin-deficient cells is unlikely to be due to altered force transmission to the nucleus. In contrast, the softer cytoskeleton would result in an underestimation of the nuclear stiffness. Based on these findings we conclude that lamin A/C-deficient cells have decreased nuclear stiffness and altered nuclear mechanics. Cellular response to mechanical strain. Strain-induced damage to the more fragile nucleus could provide one explanation for tissue-specific effects of lamin A/C mutations, for example, in mechanically active tissues like myocardium and skeletal muscle. To examine nuclear envelope integrity, we monitored the subcellular location of fluorescently labeled 70-kDa dextran microinjected into either the cytoplasm or nucleus of adherent fibroblasts. Cytoplasmic injection revealed that the high-molecular-weight dextran was excluded from the nucleus in both WT and lamin-deficient cells (Figure 3, a and b), indicating that nuclear integrity is not significantly impaired in Lmna−/− cells under resting conditions. These results were confirmed by transfecting the cells with a DsRed/peroxiredoxin-2 fusion protein. The protein lacks a nuclear localization sequence and is too large (mol wt 57 kDa) to passively diffuse into the intact nucleus. Consequently, the fusion protein was excluded from the nucleus of WT and Lmna−/− cells.

However, when dextran was injected directly into the nucleus at low and medium injection pressures (10–500 hPa), nuclear integrity in lamin A/C−deficient cells was at least temporarily compromised, resulting in fluorescently labeled dextran escaping into the cytoplasm (Figure 3d). In contrast, dextran was confined to the nucleus in most WT cells when injected at low pressure (Figure 3c), and even at medium pressure significantly more WT cells than Lmna−/− cells maintained their nuclear integrity. Using sufficiently high pressure (1,500 hPa), both WT and lamin-deficient nuclei could be ruptured (Figure 3e).

To determine whether Lmna−/− fibroblasts are thus more susceptible to mechanical strain, cells were subjected to cyclic biaxial strain (10% strain at 1 Hz). After 24 hours of strain, Lmna−/− fibroblasts had a significantly increased fraction of dead (i.e., propidium iodide–positive) cells compared with controls not subjected to strain (Figure 4a). Differences within controls (WT and Lmna−/−) and between WT control and lamin-deficient cells were not significant. Dual labeling with propidium iodide and an FITC-conjugated annexin V antibody revealed that the decrease in viability was due...
to an increase in both necrotic and apoptotic cell fractions compared with WT cells (Figure 4b).

Interestingly, when cells were injected with the fluorescently labeled 70-kDa dextran into the cytoplasm and subjected to either constant (30% for 30 minutes) or cyclic (10% at 1 Hz for 24 hours) biaxial strain, strain application did not significantly increase the number of dextran-positive nuclei in either WT or Lmna–/– fibroblasts (data not shown), suggesting that the extreme event of nuclear rupture under mechanical strain occurs in only a small fraction of cells that cannot be detected based on a small number (n = 20–30) of single cell observations.

The increased fraction of apoptotic cells in mechanically strained Lmna–/– fibroblasts indicates that necrosis through nuclear rupture can only partly explain the increased sensitivity to mechanical stimulation. Therefore, we investigated the cellular response to mechanical stimulation in more detail. Interestingly, expression of the mechanosensitive genes egr-1 and iex-1 in response to mechanical stimulation was impaired in Lmna–/– cells (Figure 4c), whereas expression of the mechanically unresponsive genes thioredoxin-1 and GAPDH was unaltered (data not shown), indicating that transcription is not impaired in a nonspecific manner.

To test whether the observed changes in Lmna–/– cells were specific to mechanical stimulation or represented a more general deficiency in signal transduction, we measured the expression levels of iex-1 and egr-1 in response to stimulation with PMA or the cytokine IL-1β. Cytokine stimulation led to an attenuated response in iex-1 but not egr-1 expression in Lmna–/– cells, while PMA responsiveness remained intact in Lmna–/– cells (Figure 4d).

NF-κB signaling. Because iex-1 is an NF-κB–dependent survival gene (23), and because NF-κB can be biomechanically activated (26), we examined whether biomechanical signaling through NF-κB is disturbed in Lmna–/– cells. The MAPK ERK1/2 is an important regulator of mechanically induced gene expression and has been linked to NF-κB activation (27). Analysis of MAPK phosphorylation after chemical (PMA) or mechanical stimulation revealed no differences between Lmna–/– and WT cells (data not shown), indicating that the observed changes are caused by alterations in signal transduction other than impaired cytoplasmic MAPK activation.

In resting cells, NF-κB is sequestered in the cytoplasm by the inhibitor IκB. Upon stimulation, IκB is ubiquinated and degraded, allowing NF-κB to translocate into the nucleus and activate target genes. Figure 5a shows that cytokine-induced cytoplasmic degradation of IκBα and translocation of the NF-κB subunit of the mechanosensitive genes egr-1 and iex-1 in response to mechanical stimulation was impaired in Lmna–/– cells (Figure 4c), whereas expression of the mechanically unresponsive genes thioredoxin-1 and GAPDH was unaltered (data not shown), indicating that transcription is not impaired in a nonspecific manner.
cells was further confirmed by nuclear microinjection experiments, which demonstrated increased nuclear rupture at low and moderate pressures compared with that in WT cells. The markedly increased nuclear deformability and fragility in Lmna–/– cells compared with WT cells has important implications for the cellular response to mechanical strain. Impaired nuclear stability can lead to rupture of the nucleus, resulting directly in cell death. Evidence of fragmented nuclei has been reported in skeletal muscle fibers from emerin-deficient Emery-Dreifuss muscular dystrophy patients and in fibroblasts from Dunnigan-type familial partial lipodystrophy patients following heat shock treatment (19, 29). This direct effect of impaired nuclear stability is also consistent with the impaired cell viability after cyclic strain observed in our experiments.

In addition to the direct effect of nuclear rupture, altered nuclear mechanics can affect cells through impaired nuclear mechanotransduction. Recent studies have reported desmin intermediate filament–mediated changes in chromatin in response to mechanical strain, and hypothesized that stretch-induced changes in chromatin can lead to activation of hypertrophy-associated genes (30). Mechanical connections between integrins, cytoskeletal filaments, and the nucleus have also been demonstrated by micromanipulation with microbeads and micropipettes in endothelial cells (31). Our nuclear strain experiments demonstrate that external strain application results in increased nuclear strain, indicating mechanical coupling between the extracellular matrix and the nucleus mediated through focal adhesion sites and the cytoskeleton. Mutations in nuclear envelope proteins such as lamin or emerin could interrupt some of these connections and impair nuclear mechanotransduction pathways. In addition, the impaired nuclear and cytoskeletal mechanics observed in Lmna–/– cells has also been demonstrated by micromanipulation with microbeads and micropipettes in endothelial cells (31).

Discussion

Our results establish the importance of lamin A/C for nuclear stability and highlight its role in transcriptional regulation in response to mechanical or chemical stimulation. Measurements of nuclear mechanics were obtained using a novel technique to measure nuclear deformation with biaxial strain applied to the cells. This method yields quantitative measurements of nuclear stiffness compared with cytoskeletal stiffness in living cells without having to isolate the nuclei. Our results for nuclear deformation in strained Lmna–/– cells are in excellent agreement with measurements obtained by Caille et al. (25) in endothelial cells under uniaxial strain and on isolated endothelial cell nuclei (28). Increased nuclear fragility in Lmna–/– cells was further confirmed by nuclear microinjection experiments, which demonstrated increased nuclear rupture at low and moderate pressures compared with that in WT cells. The markedly increased nuclear deformability and fragility in Lmna–/– cells compared with WT cells has important implications for the cellular response to mechanical strain. Impaired nuclear stability can lead to rupture of the nucleus, resulting directly in cell death. Evidence of fragmented nuclei has been reported in skeletal muscle fibers from emerin-deficient Emery-Dreifuss muscular dystrophy patients and in fibroblasts from Dunnigan-type familial partial lipodystrophy patients following heat shock treatment (19, 29). This direct effect of impaired nuclear stability is also consistent with the impaired cell viability after cyclic strain observed in our experiments.
However, increased nuclear fragility can only partially explain the phenotypes observed in several laminopathies. Even though nuclear mechanics are clearly impaired in the vast majority of Lmna−/− cells, only a very small fraction of cells (about 3–5%) exhibited nuclear rupture under strain in our experiments, and only a small number of ruptured nuclei are found in affected muscle tissue in patients suffering from Emery-Dreifuss muscular dystrophy (29). In addition, fibroblasts obtained from lipodystrophic patients with the lamin R482Q/W mutation also exhibit defective nuclear mechanics, even though these patients lack a muscular phenotype (19). Therefore, most cells appear to be functional despite distorted nuclear shape and altered nuclear mechanics, and additional events might be necessary to trigger nuclear failure. Therefore, defective nuclear mechanics could play a more important role in muscle tissue that is subjected to large mechanical strain and stress compared with (for example) adipose tissue, with cumulative cellular damage through both direct nuclear rupture and impaired mechanotransduction signaling, eventually leading to the muscular dystrophy observed in several laminopathies.

In addition to their role as nuclear envelope proteins, laminas form stable structures within the nucleoplasm as shown by fluorescence recovery after photobleaching (FRAP) (32). The impaired response of iex-1 and egr-1 expression to mechanical and cytokine stimulation, as well as the attenuated response of NF-κB–regulated luciferase activity in Lmna−/− fibroblasts despite increased transcription factor binding, indicate an important role of lamin A/C in transcriptional activation. Interactions between lamin and nuclear transcription factors have been previously demonstrated in vivo and in vitro (33, 34), and lamin A/C speckles can mediate spatial organization of splicing factor compartments and RNA polymerase II transcription (35). Disrupting the normal organization of nuclear laminas by expression of a dominant-negative lamin mutant has been shown to inhibit RNA polymerase II–dependent transcription in mammalian cells and active embryonic nuclei from Xenopus laevis (36). The impaired transcriptional activation observed in our experiments indicates a yet-unknown role of lamin A/C in the assembly of enhancosomes or as a scaffolding protein for transcription factors and coactivators.

NF-κB is a mechanical stress–responsive transcription factor that can function as an antiapoptotic signal. Impaired transcriptional activation can therefore lead to increased apoptosis in mechanically strained tissue. Furthermore, direct evidence for the role of lamin in apoptosis has previously been demonstrated in cultured cells that expressed an uncleavable mutant form of lamin (37). In these cells, chromatin failed to condense and DNA cleavage was delayed despite the activation of caspases. Kumar et al. demonstrated in ex vivo experiments that mechanical stress activated NF-κB in skeletal muscle fibers and that this response is altered in mdx mice, a model for Duchenne muscular dystrophy, stressing the importance of NF-κB signaling in muscle tissue that is affected most often in laminopathies (38).

Interestingly, several cells such as lymphoblasts, basal skin cells, and early embryonic cells do not express lamin A/C. We speculate that nuclear mechanics and mechanotransduction in these cells would be normal, since these cells are known to be capable of normal function. In these cells, the role of lamin A/C might be taken over by a different protein or complex of proteins. However, it is important to note that our experiments used only fibroblasts, and future studies of cells from different tissues are essential.

While all experiments were performed in fibroblasts that completely lack lamin A and lamin C, most human diseases arise from heterozygous lamin mutations. In these situations mutant lamin often appears as stable as WT lamin and is expressed at similar levels (39). In many cases, especially in phenotypes involving striated muscle, the mutation may lead to a structurally impaired form of lamin A/C that could act as a dominant negative and lead to cellular mechanical deficiencies as observed in the lamin A/C–null cells. Other mutations, such as those resulting in lipodystrophy, could affect the binding of lamin to other proteins or chromatin with fewer effects on the structural role of lamin itself, resulting in a partially functional protein that might affect only specific signaling pathways. Furthermore, combinations of mechanical and transcriptional regulation defects could result in complex phenotypes affecting several tissue types in diseases such as progeria. By providing independent tests for measuring structural and gene-regulatory functions of lamin A/C, our experiments could help distinguish the effects of individual mutations on the function of lamin.

Tissue-specific effects observed in several laminopathies may thus arise from two mechanisms: the impaired nuclear stability renders mechanically strained tissue more susceptible to cellular damage, and abnormal transcriptional activation impairs adaptive and protective pathways. Individual mutations in the lamin A/C gene could potentially selectively interfere with any of these functions, explaining the diversity of observed phenotypes.