Matrix Elasticity Regulates Lamin-A,C Phosphorylation and Turnover with Feedback to Actomyosin

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Report

DOI: http://dx.doi.org/10.1016/j.cub.2014.07.007

Highlights

* MSCs on soft matrix exhibit a less spread nucleus with low lamin-A,C levels
* Lamin-A,C is rapidly phosphorylated in response to reduced cytoskeletal tension
* Phosphorylation leads to nuclear softening and lamin-A,C turnover
* Levels of lamin-A,C and myosin-IIA are coregulated in response to matrix elasticity

Summary

Tissue microenvironments are characterized not only in terms of chemical composition but also by collective properties such as stiffness, which influences the contractility of a cell, its adhesive morphology, and even differentiation [1–8]. The nucleoskeletal protein lamin-A,C increases with matrix stiffness, confers nuclear mechanical properties, and influences differentiation of mesenchymal stem cells (MSCs), whereas B-type lamins remain relatively constant [9]. Here we show in single-cell analyses that matrix stiffness couples to myosin-II activity to promote lamin-A,C dephosphorylation at Ser22, which regulates turnover, lamina physical properties, and actomyosin expression. Lamin-A,C phosphorylation is low in interphase versus dividing cells, and its levels rise with states of nuclear rounding in which myosin-II generates little to no tension. Phosphorylated lamin-A,C localizes to nucleoplasm, and phosphorylation is enriched on lamin-A,C fragments and is suppressed by a cyclin-dependent kinase (CDK) inhibitor. Lamin-A,C knockdown in primary MSCs suppresses transcripts predominantly among actomyosin genes, especially in the serum response factor (SRF) pathway. Levels of myosin-IIA thus parallel levels of lamin-A,C, with phosphoiso mutants revealing a key role for phoshoregulation. In modeling the system as a parsimonious gene circuit, we show that tension-dependent stabilization of lamin-A,C and myosin-IIA can suitably couple nuclear and cell morphology downstream of matrix mechanics.
Matrix Elasticity Regulates Lamin-A,C Phosphorylation and Turnover with Feedback to Actomyosin

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Summary

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Results and Discussion

Lamin-A,C Phosphorylation in Interphase Cells Is Favored by Low Nuclear Stress

The inner nuclear membrane is lined by juxtaposed networks of two types of intermediate filament proteins. The main products of the LMNA gene, lamin-A and the truncated spliceform lamin-C, have long been known to vary greatly between different tissues [10]. LMNB1 is the founding member of the intermediate filament superfamily [11] that also includes LMNB2, which varies minimally in expression between different tissues [9]. The lamina interacts with numerous nuclear proteins and chromatin, as well as links across the nuclear envelope to the cytoskeleton [12–14]. Our recent proteomics analyses of tissue samples and cells showed that lamin-A,C (LMNA) is unique among these various factors in increasing systematically with tissue stiffness [9]. We further showed with cultured cells that lamin-A,C increases with matrix stiffness and can enhance differentiation, although the molecular basis for mechanoregulation was unclear. Mass spectrometry (MS) of bulk lysates suggested that lamin-A,C was more phosphorylated on soft matrices than stiff matrices while B-type lamins showed no detectable phosphorylation. All lamins are abundantly phosphorylated in rounded mitotic cells (uncaging the chromatin). One of the best-characterized phosphorylation sites in lamin-A,C is Ser22 [15, 16], for which there is an antibody suitable for high-resolution cell imaging. We therefore hypothesized that pSer22 in individual interphase cells would be highest in states of low cell tension.

Quantitative immunofluorescence showed pSer22 in the nuclei of every cell (Figure 1A and Figures S1A–S1C available online), amounting to ~5%–10% of total lamin-A,C as calibrated by synthetic peptides using MS (Figures 1B, S1D, and S1E). Intensities in each interphase nucleus were at least several fold above the intensity of the secondary antibody control (Figure S1B) while also being ~10-fold less than those in dividing cells (Figures 1B, S1C, and S1D). Specificity of anti-pSer22 was confirmed with an epitope blocking phosphopeptide that greatly decreased signal in both nondividing nuclei and dividing cell cytoplasm (Figure 1B). Lamin-A,C is thus phosphorylated at Ser22 during interphase. The A549 cell line used in these initial imaging studies possesses some key mesenchymal characteristics [17], but similar observations apply to primary human MSCs that are well known for their contractility-modulated adhesion [2].

Mesenchymal stem cells (MSCs) were seeded from suspension onto soft (0.3 kPa) or stiff (40 kPa) collagen-coated matrices and were then fixed and imaged at various time points. The fraction of lamin-A,C phosphorylated at Ser22 (pSer22/LMNA) decreased concomitantly with greater nuclear spreading and total lamin-A,C, which were both promoted by stiff matrix (Figure 1C). Cells cultured on a very thin layer (3–15 μm) of soft matrix on top of glass exhibited behavior intermediate between that of cells on soft and stiff gels (both >35 μm thick), and indeed in the case of total lamin-A,C and pSer22 per LMNA, the thin, soft gel behaved most like the thick, stiff gel. The thin, soft gel had the same chemical composition as the thicker soft gel, but the proximity of the hard glass substrate increases the apparent stiffness “felt” by the cell so that hydrogel composition seems unimportant [18, 19]. After 24 hr, lamin-A,C dephosphorylation on soft gels was one-half of that on stiff matrices, but changes in phosphorylation were observed within hours of cell adhesion, which is similar in time scale to changes in lamin-A,C level and nuclear spreading. Rapid posttranslational changes under stress have been reported in other mechanotransduction pathways such as p130Cas when extended by cell tension to expose sites for phosphorylation [4] (opposite to the trend here). For lamin-A,C in isolated nuclei, we have recently demonstrated stress modulation of a site-specific, nonenzymatic modification (i.e., fluorophore conjugation to a buried cysteine) [9], and so stress modulation of lamin-A,C phosphorylation could...
Figure 1. Increased Stress on the Nucleus Suppresses Lamin-A,C Phosphorylation

(A) Lamin-A,C pSer22 is present in interphase cells. As shown in the top two rows, confocal image stacks of total and pSer22 lamin-A,C in MSCs fixed after 1–24 hr of adhesion showed wrinkled nuclei at early stages of cell adhesion that stretched and smoothed with spreading. The third row shows pSer22/LMNA ratios from the top two rows calculated pixel by pixel and normalized to the mean fold change. After 24 hr, a greater phospho-LMNA concentration was observed in the nucleoplasm versus the nuclear periphery. The bottom row shows confocal cross-sections that confirmed nucleoplasmic pSer22. Scale bars, 10 μm.

(B) Histogram of pSer22 levels in a population of A549 cells (Figures S1 A–S1D) showing specificity of pSer22 immunofluorescence and stoichiometry calibrated by MS (Figure S1E). Dividing cells showed the greatest extent of phosphorylation (Figure S1C). Preincubation with a phosphoepitope-blocking peptide decreased immunofluorescence intensity, as did nonspecific binding to a nonphosphorylated version of the same peptide, but to a significantly lesser extent (Figure S1D). In the absence of primary antibody (second-degree antibody only), fluorescence intensity was very low. n = 33–249 cells per group.

(legend continued on next page)
likewise depend on stress modulation of lamin-A,C’s structure. However, here we focus on the important downstream consequences of phosphorylated lamin-A,C after providing further evidence of a stress-modulated mechanism. Upon detachment from substrate into suspension, MSCs and their nuclei rapidly rounded as cytoskeletal tension was relaxed. This process was accompanied within tens of minutes by lamin-A,C phosphorylation at Ser22 (Figure 1D). Cytoskeletal tension was similarly relaxed by treatment with the myosin-II inhibitor blebbistatin (blebb), resulting in a reduction in nuclear spread area and increased pSer22/LMNA (Figures 1E and S1E). Knockdown (KD) of myosin-IIA also increased pSer22/LMNA (Figure S1F), and expression of a phosphomimetic myosin-IIA construct, S1943D, known to suppress stress fiber assembly [20, 21] lowered the amount of lamin-A,C (Figure S1G). Matrix mechanics, cell detachment or reattachment, cell spreading, and myosin inhibition all collectively and independently support the conclusion that low nuclear stress favors lamin-A,C phosphorylation.

A nominal “nuclear tension” in cell spreading was estimated from the product of nuclear strain and nuclear stiffness, as calculated respectively from the fold change in projected nuclear area (Figure 1F) and from the level of lamin-A,C, which contributes to stiffness (see the Supplemental Information). Replotting of pSer22/LMNA versus matrix-dependent MSC spreading (Figure 1C) and blebb treatment (Figure 1E) versus nuclear tension collapsed all of the data onto hyperbolic decays, consistent with inhibition of lamin-A,C phosphorylation by nuclear tension (Figures 1G and 1H).

Lamin-A Phosphomutants Moderate Nuclear Mechanosensitivity

For assessment of the functional importance and properties of lamin-A,C phosphorylation, MSCs were transfected with GFP fusions of lamin-A having either a phosphomimetic S22D or a nonphosphorylatable S22A and compared to cells transfected with WT GFP-lamin-A. Fluorescence recovery after photobleaching (FRAP; Figure 2A) was used to assess the mobile fractions, f, (at 5–10 min) of the S22 variant proteins during and after cell attachment to soft and stiff substrates. FRAP was started 30 min after plating of MSCs, and blebb was added after 24 hr. Up to 30%–40% of WT GFP-lamin-A was mobile 30 min after plating, regardless of matrix stiffness (Figure S2A). However, lamin-A was progressively immobilized with cell adhesion, and by just 2 hr (with $t = 1$ hr) the soluble fraction was a stable ~15% on soft matrix and <5% on stiff matrix. The higher mobility of WT GFP-lamin-A in rounded nuclei on soft matrix at both 2 hr and 24 hr, and also after inhibition of contractility with blebb treatment (Figure 2B), was consistent with higher pSer22 (Figures 1C and 1E). These findings are also consistent with the fact that during cell division the phosphorylation of Ser22 solubilizes lamin-A,C [15].

In contrast to WT lamin-A, neither S22D nor S22A exhibited a dependence of the mobile fraction, f, on matrix stiffness (Figures 2B and S2B). Phosphorylation of lamin-A thus appeared to be downstream of matrix. Moreover, S22D mobility remained high 4-fold longer than the WT ($t$ = 4 hr), regardless of stiffness. Although by 24 hr f was similar for S22D and the WT on soft gels, perhaps due to phosphorylation of sites other than Ser22, blebb had no significant effect on f for S22D. Since S22A lamin-A was completely immobilized after only 2 hr of adhesion to any matrix (Figure S2B), the phosphodynamics of Ser22 seemed important to lamin-A,C mobility. A similar increase in mobile fraction for phosphomimetic S22E lamin-A over the WT and S22A was observed in lung carcinoma A549 cells (Figure S2C).

While ectopically expressed WT GFP-LMNA localized predominantly at the nuclear envelope, as did S22A, the phosphomimetic S22D appeared to be more homogenous within MSC nuclei (Figure 2C). A diffuse nuclear distribution of S22D was consistent with confocal z stack imaging that showed nucleoplasmic pSer22 staining in fixed MSCs (Figure 1A, bottom). Live-cell imaging showed that both S22D and S22A completely softened micropipette-aspirated nuclei at fixed stress showed softening of S22D nuclei and stiffening with S22A relative to the WT. The phosphodynamics of S22 in lamin-A,C are thus critical in determining the structural organization and mechanics of nuclei during cell spreading.

Lamin-A,C Degradation Is Downstream of Phosphorylation

During cell division, comprehensive phosphorylation is required to disassemble the lamina, but during interphase, our results suggest that cells titrate lamin-A,C levels and phosphorylation to regulate molecular mobility and nuclear stiffness in proportion to cytoskeletal stress and matrix stiffness. We hypothesized that these processes were linked, with high pSer22 in relaxed cells favoring lamin-A,C degradation.
Full-lane immunoblots of lamin-A,C in MSCs maintained in suspension in serum-supplemented media for 10 or 45 min showed a decrease in intact lamin-A,C with time and slightly increased intensity of weak lower bands (Figure 3A). Immunostaining of the same blots for pSer22 revealed the same bands plus many additional lower bands, with densitometry illustrating the differences (Figures 3B, S3A, and S3B). So that specificity of the antibody could be confirmed, gels were sliced for MS analyses, and the lower-molecular-weight (MW) bands indeed yielded up to 28 distinct lamin-A,C-derived peptides (Figures 3C, 3D, and S3C). Lamin-A,C peptides were found in each of the lower-MW gel slices from lysates of MSCs and A549 cells (Figures 3D and S3D). Immunoblotting with antibody against cleaved lamin-A also revealed an abundance of signal at low MW with very little signal from intact protein (Figure 3E). Immunoblot analyses—as with any bulk technique—could reflect distinct subpopulations of cells, but immunofluorescence imaging demonstrated that anti-pSer22, as well as monoclonal and polyclonal antibodies against anti-cleaved lamin-A, produced significant signal in every nucleus (Figure 3F).

To assess whether degradation could be downstream of phosphorylation, we leveraged the fact that lamin-A,C is classically a target of cyclin-dependent kinases (CDKs). MSCs were treated with an inhibitor, RO3306, shown to act against several CDKs at μM concentration [23]; treatments of ≤6 hr tend to minimize extraneous changes in protein levels. In drug-treated cells, quantitative immunofluorescence revealed a ~50% decrease in pSer22 and >80% decrease in immunostaining of cleaved lamin-A,C, as assessed with two independent antibodies (Figures 3F, 3G, and S3F). Drug-treated cells also showed higher lamin-A,C and larger nuclear areas, consistent with a high-contractility phenotype induced by phosphoinhibition (Figure S3G). Additionally, plotting of pSer22/LMNA as a function of nuclear tension (as in Figures 1G and 1H) fit to an appropriate hyperbolic form (Figure S3H). Since the CDK inhibitor suppressed lamin-A,C phosphorylation and degradation, we expected that drug-treated nuclei would also be stiffer in micropipette aspiration; indeed, the nuclear relaxation times were ~10,000-fold longer than untreated cells (Figure 3H). Phosphorylation of lamin-A,C thus precedes turnover and nuclear softening.

Correlated Phosphorylation of S390 and S22 Sites in Lamin-A,C

A large number of lamin-A,C phosphorylation sites have been identified by MS, with many occurring in mitosis [24–26]. Our earlier MS studies identified Ser22, Ser390, Ser404, and Thr424 as more phosphorylated on soft compared to stiff substrate [9], and recent work has associated phosphorylation of
Figure 3. Phosphorylation of Lamin-A,C Promotes Proteolysis and Nuclear Softening

(A) Phospho-lamin-A,C is present in both full-length and cleaved states. MSCs lysed after short (10 min) and long (45 min) periods of rounding in suspension showed numerous low-MW, fragmentation-product bands in immunoblots against lamin-A,C and, to a greater extent, against pSer22.

(B) Profiles of immunoblots showing MW ranges analyzed by MS (densitometry shown in Figures S3A and S3B).

(C) Gel slices A–D were analyzed by MS, confirming the existence of low-MW lamin-A cleavage products (peptide coverage of 25–40 kDa range is shown; blue-to-red coloring indicates peptides detected with increased ion current). Phosphorylation sites detectable by MS are indicated in yellow.

(D) All examined bands had lamin-A fragments (see Figure S3C for sequence coverage maps).

(E) Inhibition of lamin-A,C phosphorylation suppresses cleavage. Immunoblots against lamin-A pSer22 or lamin-A cleavage product(s) are shown. The latter antibody shows minimal detection of intact lamin-A and a high-intensity band at 40 kDa.

(F) Treatment with CDK inhibitor RO3306 reduced the extent of phosphorylation at Ser22 in MSCs.

(G) RO3306 also suppressed the formation of lamin-A,C cleavage products as determined by two independent antibodies (see Figures S3F–S3H for representative images and analysis).

(H) Drug-induced lamin-A,C phosphoinhibition stiffened the nucleus as determined by micropipette aspiration (relaxation times determined at constant compliance, $J = 1.25$ kPa $^{-1}$ per [22]). Error bars show the log mean ± SEM. n > 4 nuclei.

(I) MS showed a correlated increase in phosphorylation at S22 and S390 during lamin-A overexpression in A549 cells.

(J) Phosphorylation at S22 and S390 showed a correlated decrease during lamin-A,C KD in MSCs. Error bars show ±SEM. (See Figure S1D for calibration of phosphorylation measurements by MS.) Error bars indicate ±SEM. See also Figure S3.
Figure 4. Lamin-A,C Level and Phosphorylation State Regulate Myosin-IIA, Factors that Can Be Combined into a Mechanosensitive Gene Circuit Model

(A) Lamin-A,C level regulates myosin-IIA through the serum response factor (SRF) pathway. Transcriptional profiling of MSCs subjected to lamin-A,C KD is shown (n = 3) versus nontreated wild-type control (NT or WT, equivalent). SRF and cofactors (in cyan) and target genes (blue) were significantly suppressed. The YAP1-regulated Hippo pathway has also been implicated in cellular mechanosensitivity [7], but it appeared to be unaffected here. (B) Transcripts of SRF and cofactors (cyan) were suppressed with lamin-A,C KD, along with those of multiple actin-binding nuclear envelope proteins (red) (n = 3). (See Figure S4A for Gene Ontology term analysis.) (C) Correlation between protein and transcript changes with lamin-A,C KD in MSCs. Points represent 485 proteins quantified by MS with three or more peptides per protein, and their associated mRNA was quantified by DNA microarray. Each point is averaged from three biological replicates, and the transcripts or proteins that are significantly perturbed (p < 0.05) are counted in the four quadrants of the plot. Of genes with Gene Ontology annotation “actin cytoskeleton,” (n = 52, in red), 14 are unchanged and 36 have reduced levels of protein and mRNA. Of genes classified as SRF targets (n = 12) by Olson et al., four are unchanged and eight—including myosin-IIA (MYH9)—have reduced levels of protein and mRNA [31].

(legend continued on next page)
Ser404 with turnover of lamin-A precursor [27]. While the well-studied pSer22 modification is recognized by commercial antibodies, MS analyses of multiple samples showed that overexpression of lamin-A resulted in greater phosphorylation of both Ser22 and Ser390 (Figure S1E). The addition here of excess lamin-A, perturbing the mechanical equilibrium of the cell and conceivably relieving or disrupting tension on the nucleuskeleton, is perhaps compensated for in the cell by increased phosphorylation and subsequent lamin turnover. Conversely, lamin-A,C KD resulted in reduced phosphorylation at both sites, thus helping to maintain the integrity of the diminished lamina (Figure S5J). Furthermore, titration with synthetic peptides showed Ser390 to be phosphorylated 1%-5% in both A549 cells and MSCs (Figure S1E). Importantly, the same pSer390 phosphopeptide was also detected in tryptic peptides from lysates of multiple mouse tissues (brain, muscle, and heart) [5], but the numerous cell types in such tissues complicate interpretations and strongly motivate the studies of specific human cell types here.

Lamin-A,C Level and Phosphomutants Regulate Myosin-IIA

Since the phosphomimetic mutant of lamin-A,C led to nuclear rounding and lamin-A,C interacts with nuclear actin and actin-binding proteins [23, 29] to partially regulate at least a few components in the serum response factor (SRF) pathway [9, 30], we hypothesized broad control of actomyosin gene expression [31]. Whole-genome transcriptional profiling of LMNA KD MSCs showed repression of target genes and cofactors of the mechanosensitive SRF pathway (Figures 4A and 4B), leading to broad suppression of the actin cytoskeleton to a greater extent than in any other pathways (Figure S4A). Proteomic profiles showed excellent correlation with transcript profiles, with all significantly altered SRF targets, including myosin-IIA (MYH9 gene), being repressed at both the protein and mRNA level (Figure 4C).

To investigate a functional link between lamin-A,C phosphorylation and myosin-IIA (MYH9), we established A549 cell lines with small hairpin RNA knockdown of endogenous lamin-A,C plus transduction with GFP-lamin-A constructs WT, S22E, and S22A (Figures 4D and S4B–S4D). The phosphomimetic showed in the immunoblot a lower-MW band consistent with Figure 3E and phosphorylation-initiated degradation. Overexpression of lamin-A generally increased myosin-IIA above a basal level (Figure 4E), and at higher overexpression levels of the nonphosphorylatable and immobile S22A construct, myosin-IIA increased to a much greater extent compared to overexpression of phosphomimetic S22E. A hyperbolic fit to the S22A data intercepted the y axis at a nonzero value, suggesting that a fraction of myosin-IIA expression is independent of lamin-A,C, which is consistent with the presence of this essential myosin in lamin-A,C knockout mice [32, 33]. Since lamin-A,C primarily regulates the actomyosin cytoskeleton, depolymerization of F-actin in our studies of nuclear stiffness (see the Experimental Procedures) as modulated by lamin-A,C phosphorylation (Figures 2E and 3H) seems likely to reveal properties attributable directly to the structural state of lamin-A,C.

Systems Mechanobiology Gene Circuit Couples Lamin-A,C and Myosin-IIA Expression

While our recent work established that lamin-A,C positively regulated its own transcription factor [9], lamin-A,C phosphodynamics here affect also myosin-IIA levels. Based on these and additional experimental insights, a parsimonious model of expression and degradation for both lamin-A,C and myosin-IIA message and protein was formulated as a “mechanobiological gene circuit” (MGC) (Figure 4F and the Supplemental Information) in order to assess whether a stably coupled system could be reasonably achieved. The most important and atypical aspect of the model is mechanically regulated degradation of these two structural proteins, which follows otherwise standard cooperative Michaelis-Menten type enzyme kinetics. Transcription of both genes was assumed to be linear in lamin-A,C protein, and linearity was also assumed for degradation of transcript as well as for protein synthesis. Equations describing the MGC were solved numerically, constrained by experimental observations (Figures 1G and 1H and the Supplemental Information), and the nonlinear degradation terms fed back into the gene circuit to generate stable expression states of both lamin-A,C and myosin-IIA (Figure 4G). As matrix stiffness and cell tension suppressed protein phosphorylation and turnover (modeled from relations in the MGC in Figure 4H), steady-state levels monotonically increased with matrix E, consistent with coupled mechanoregulation of lamin-A,C and myosin-IIA. With this initial model in hand, further experiments will be needed to measure the stress-dependent rate constants for the lamins and many other relevant proteins. Next-generation, high-throughput methods of sequencing and proteomics seem highly appropriate [34] for these microenvironment-dependent metabolic questions.

Experimental Procedures

Additional details of the isolation and culture of primary MSCs, cell treatments and phosphomimetic constructs, immunofluorescence and confocal imaging, FRAP, immunoblotting, transcriptional profiling, and
micropipette aspiration can be found in the Supplemental Experimental Procedures.

Preparation of Soft and Stiff Hydrogel Substrates

The preparation of polyacrylamide gels with controlled elasticity and covalent attachment to glass coverslips was described in detail in a published methods paper [18]; the method is also summarized in the Supplemental Experimental Procedures.

MS and Quantification of Synthetic Phosphopeptides

Quantification of proteins by label-free MS is described in the Supplemental Experimental Procedures and earlier work [36]. MS response to phosphorylation at S22 and S390 was calibrated using synthetic versions of tryptic peptides (SGAQASSTPLSPTR, SGAQASSTPL[pSer22]PTR, and LRLSP SPTSPQR LRL[pSer390]SPTSPQR; GenScript), which were spiked into a tryptic cell digest (60–80 kDa MW band) (Figure S1E). The data in Figures 3C, 3D, and S3C–S3E were attained without alignment between spectra (Blundell, Rosetta Biosystems). The data in Figures II and III were aligned against standards containing both phosphorylated and unphosphorylated versions of the S22 and S390 tryptic peptides. Specificity of primary antibody was verified using standards containing both phosphorylated and unphosphorylated tryptic cell digests (60–80 kDa MW band) (Figure S1E). The data in Figures 4G and 4H were aligned by subtractive proteomics (SGAQASSTPL[pSer22]PTR) at a peptide:antibody ratio of 10:1 (Figures 1B and S1D).

Systems Mechanobiology Gene Circuit

Derivations of the equations used to describe the interactions in Figure 4F are shown in the Supplemental Information and are solved in the steady state to produce the data plotted in Figures 4G and 4H using code written in Mathematica (Wolfram).

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.07.001.

Author Contributions


Acknowledgments

We are grateful for support from the US NIH (grants R01HL062352, P01DK032094, R01EB007049, P01DK090969, and NCATS-BUL1TR000003), the US National Science Foundation (grant 1208854), an American Heart Association Grant in Aid (14GRNT20490285), the Human Frontier Science Program, and the University of Pennsylvania’s research centers (Materials Research Science and Engineering; Nano Science and Engineering; Nano/ Bio Interface).

Received: April 15, 2014
Revised: May 28, 2014
Accepted: July 1, 2014
Published: August 7, 2014

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Please cite this article in press as: Buxboim et al., Matrix Elasticity Regulates Lamin-A,C Phosphorylation and Turnover with Feedback to Actomyosin, Current Biology (2014), http://dx.doi.org/10.1016/j.cub.2014.07.001
Supplemental Information

Matrix elasticity regulates lamin-A,C phosphorylation and turnover with feedback to actomyosin

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Supplemental figures

Figure S1.

A) Lamin-A and Lamin-C phosphorylation and actomyosin: supplemental materials

B) Increased exposure time

C) DNA

D) Endogenous phosphorylation in A549s (60-80 kDa)

E) Non-dividing cells

F) WT

G) siMIIA

H) WT
Figure S1.

pSer22 immunostain of all interphase nuclei is > 2° antibody (Ab) control, but << dividing cells (see Figs. 1A, B).

(A) Immunostaining specificity to the pSer22 epitope was demonstrated in A549 cells by comparison to secondary antibody-only staining, in conjunction with staining for lamin-A,C (LMNA) that was imaged in a different channel. Note that the exposure time is increased in the bottom-right panel to show that non-specific binding is not localized to the nucleus. (B) Antibody binding to pSer22 was found to be four-fold higher than secondary-only non-specific binding. (C) Antibody specificity to the phosphorylated epitope was confirmed by a higher pSer22 immunostaining intensity in cells undergoing mitosis.

**Calibration of endogenous pSer22 using immunostaining and calibrated mass spectrometry (see Fig. 1B).**

(D) Plot showing normalized pSer22/LMNA intensity ratio in dividing and non-dividing A549 cells, with addition of phosphorylated and non-phosphorylated Ser22 peptides, and a secondary-antibody only control. The Ser22 peptide showed some non-specific blocking of the pSer22 antibody, but was always significantly distinguishable from when blocking with the pSer22 peptide. (E) To evaluate pSer22/LMNA stoichiometry in interphase cells, full length (i.e. not proteolytically degraded) lamin-A,C from an A549 cell lysate was obtained from the 60-80 kDa region of an SDS-PAGE gel and interrogated by MS. The extent of phosphorylation was calibrated relative to synthetic peptides: 1-2% of lamin-A,C was found to be phosphorylated at Ser22. A similar treatment showed ~ 5% of lamin-A,C to be phosphorylated at Ser390. Immunoblotting densitometry of the entire SDS-PAGE gel lane (i.e. inclusive of proteolytic lamin fragments) showed that ~ 20% of total pSer22 was from intact, non-degraded lamin-A,C in MSCs (Figs. 3A, B; S3A, B). Assuming pSer22 stoichiometry of intact lamin-A,C to be 1-2% in MSCs, the fraction of pSer22 epitopes relative to total lamin-A,C would be 5-10%. Dividing cells (< 1% of the population) showed 10-fold higher pSer22 intensity than interphase cells (Fig. 1B), suggesting that 50-100% of lamin-A,C was phosphorylated during mitotic breakdown of the nuclear envelope (consistent with Fig. S1C).

**Effect of myosin-IIA on lamin-A,C regulation (see Fig. 1E).**
(F) Representative images of MSCs at 2 hours of adhesion, with and without blebbistatin (blebb) treatment. LMNA levels were lower in blebb treated cells, but the level of pSer22 relative to LMNA was high (quantitative image analysis is shown in Fig. 1E). (G) siRNA knockdown of myosin-IIA lead to a lower fraction of LMNA being phosphorylated at Ser22, as determined by quantitative immunoblotting. (H) In MSCs expressing a phosphomimetic S1943D mutant GFP-myosin-IIA construct, which suppresses stress-fiber formation [S1, S2], total myosin-IIA levels are the same as those in cells expressing a WT construct, but lamin-A,C is down-regulated consistent with a low contractility phenotype (n > 90 cells per group; bars show ± SEM).
Figure S2.

A

B

C

Mobile fraction, f

Adhesion time (hours)

MSCs

S22D

S22A

WT

Mobile fraction, f

n = 13-19

A549s

WT

S22A

S22E

p << 0.001

p = 0.03

p < 0.001

p < 0.001

p << 0.001
Figure S2.

Mobile GFP-lamin-A fraction in MSCs (as a function of adhesion time) and A549s (see Fig. 2).

The mobile fraction of a GFP-lamin-A construct, $f$, was evaluated by fitting an exponential curve to the intensity recovery following photobleaching (Fig. 2A). (A) $f_{WT}$ decreased with adhesion time of MSCs for the first 2 hours, reaching ~15% on soft (0.3 kPa) but less than 5% on stiff gels (40 kPa) (3-5 nuclei grouped per time point; projected nuclear areas are indicated by the color of each point). Lamin-A mobility was increased by blebb treatment after 24 hours on stiff gels. (B) Independent of matrix stiffness, S22D mobile fractions decayed three folds slower than WT, whereas S22A remained immobile. Soft matrices and blebb drive WT lamin-A solubilization, but S22D and S22A show no matrix or blebb dependence (summarized in Fig. 2B). (C) Consistent with observations in MSCs, fluorescence recovery experiments using A549 cells showed a greater mobile fraction with phosphomimetic GFP-lamin-A S22E than with WT or non-phosphorylatable S22A.
Figure S3.

Densitometry of lamin pSer22 western blots (see Figs. 3A, B).

(A, B) Mesenchymal stem cells (MSCs) were lysed following 10 or 45 minutes in suspension and analyzed by immunoblotting against lamin-A,C and pSer22 (Fig. 3A). Densitometry analysis showed that the majority of the phosphorylated lamin-A,C was fragmented below the expected molecular weight (MW; 75 kDa for lamin-A; 65 kDa for lamin-C).

Sequence coverage of lamin-A,C fragments (see Figs. 3C, D).

(C) The MSC lysate was separated by SDS-PAGE and analyzed in discrete MW ranges by MS (Fig. 3B), confirming that lamin fragments were found at lower MW. While peptide coverage clearly extended across all weight ranges, slice-D (< 25 kDa) showed an enrichment of tail-domain and Ig-fold peptides whereas slice-B (40-57 kDa) showed head and coiled-coil rod domains. Relative coverage intensity (i.e. signal strength of tryptic peptides) is indicated by the color of the sequence. Sites of phosphorylation detected by mass spectrometry are highlighted in yellow. (D) The presence of lamin-A,C fragments was also confirmed by MS in lower MW bands of A549 cell lysates. (E) MSCs were treated with a kinase-inhibitor (RO3306) and compared with non-treated control cells, with analysis performed by MS (with MW bands as shown in Fig. 3B). Changes in ion current of lamin-A,C peptides were assessed between samples. Enrichment of intermediate MW lamin fragments in RO3306-treated cells may be indicative of a phosphorylation event that is necessary for the progression of lamin cleavage.

Image analysis of cleaved lamin staining during inhibition of phosphorylation (see Figs. 3F, G).

(F) Representative images showing fixed MSCs, with and without kinase inhibitor RO3306 treatment, with immunostaining against lamin-A,C, pSer22 and cleaved lamin-A with either a monoclonal or a polyclonal antibody (data summarized in Figs. 3F, G). (G) Image analysis showed that lamin-A,C levels were ~50% higher and the projected area of the nucleus larger in drug-treated MSCs relative to non-treated cells, reminiscent of a highly contractile phenotype (n = 50-51 cells per group). (H) Cell-by-cell analysis of S22 phosphorylation in MSCs treated with kinase inhibitor RO3306 (Figs. 3F, G; S3F, G), plotted as a function of nuclear tension with hyperbolic-like fit consistent with the Mechanobiological Gene Circuit (MGC) model (Figs. 1F-H, 4E).
Figure S4.

A

<table>
<thead>
<tr>
<th>Score</th>
<th>Molecular function</th>
<th>Cellular component</th>
<th>Biological process</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1</td>
<td>Actin filament binding</td>
<td>Cytoskeleton, Actin</td>
<td>Actin filament bundle formation</td>
<td>LMNA, EZR, ACTN4, FSCN1, ACTN1,...</td>
</tr>
</tbody>
</table>
| 5.7   | Actin cytoskeleton, non-membrane-bound organell | Actin filament, cell junction | PPP4R3, LMNA, TUBB3A, ARP4, TPM2, ...
| 4.7   | Focal adhesion, cell junction | Cytoskeleton, Actin | LMNA, SYT11, NXN, PERP, TES, VCL, ...
| 3.6   | Cell cortex, Cytoskeleton | Actin filament cytoskeleton | ACTB, EZR, ACTN4, LASP1, MYH9, DSN1, ...
| 2.8   | Calponin-like actin-binding | Actin-binding | ACTN4, ACTN1, FLNA, ...
| 2.6   | Calponin-like actin-binding | Actin-binding | PPP4R3, NEDD1, KRT18, NDEL1, CALM3, ...
| 2     | Muscle myofibril, sarcomere | Muscle myofibril, sarcomere | ANK2, ACTN1, TPM2, ...
| 1.8   | Leukocyte transendothelial migration | Adherens junction | ACTB, ACTN4, ACTN1, VCL, ...
| 1.8   | Regulation of actin cytoskeleton | Focal adhesion | TAGLN, CNN2, CNN1, ...
| 1     | Regulation of kinase activity | Cytoskeleton, Actin | PPP4R2, NEDD1, KRT18, NDEL1, CALM3, ...
| 0.8   | Response to hormone stimulus | Cytoskeleton, Actin | ANK2, ACTN1, TPM2, ...
| 0.7   | Response to hormone stimulus | Cytoskeleton, Actin | ANK2, ACTN1, TPM2, ...

B

Hoechst | GFP-WT-lamin-A | LMNA | Myosin-IIA | 20 µm

C

No significant differences

D

No significant differences
Figure S4.

GO-term analysis shows that lamin-A,C KD down-regulates actin cytoskeletal genes (see Figs. 4A-C).

(A) Gene ontology term analysis of the entire transcriptome shows that the cytoskeleton is broadly affected by lamin-A,C knockdown, consistent with perturbation to the serum response factor (SRF) pathway. The ten most significant changes, as evaluated by DAVID bioinformatics resources [S3], are instances of down-regulation, with seven of the ten (including the top three) related to the actomyosin cytoskeleton.

Lamin-A phosphomutations do not significantly affect chromatin or nuclear spreading in A549s (see Figs. 4D, E).

(B) Endogenous lamin-A,C was knocked down in A549 cells in conjunction with expression of GFP-lamin-A phosphomutant constructs (WT, S22A and S22E) in order to assess the effect on myosin-IIA. Representative images showed the expected localization of GFP-lamin-A at the nuclear envelope. Quantitative image analysis showed that neither Hoechst DNA staining (C) or nuclear spread area (D) were significantly altered by lamin-A phosphomutant expression (n = 70-113 cells per group).
Supplemental materials and methods

Isolation of fresh MSCs from bone marrow

Bone marrow aspirates were obtained from posterior iliac crest of anonymous human donors (University of Pennsylvania Stem Cell Core, with Institutional Review Board approval) under the procedures and regulations defined by the Helsinki agreement. Mono-nucleated cells (MNCs) were obtained using a Ficoll density gradient (Ficoll-Paque PLUS, GE Healthcare) and depleted from CD34-positive cells by a micro-bead kit (Direct CD34 Progenitor Cell Isolation Kit, Miltenyi Biotec) and screened by automated cell separation (AutoMacs, Miltenyi Biotec) according to manufacturer’s protocols. MNCs were re-suspended in 10% FBS (Sigma-Aldrich) and 1% penicillin/streptomycin antibiotics (P/S, GE Healthcare) supplemented low-glucose basal medium (DMEM, Life Technologies). Typically, MNCs were seeded at 10-100k cells/cm² in standard tissue-culture plastic flasks and incubated at 37 °C and 5% CO2 humidified conditions. Cells were thoroughly rinsed in PBS x 3 after 24 hrs to remove non-adherent cells. Fibroblastic colony forming unit (CFU) stromal cells appeared within 3-4 days and cells expansion included medium exchange every 4 days. Cells confluence was maintained < 80% by passaging cells and re-seeding at > 50% confluence. Expression of stromal stem cells markers (CD105, CD166, CD44, CD90) and the lack of hematopoietic markers (CD45-RA, CD34) was verified by flow cytometry (data not shown). Differentiation capacity towards fat and bone was verified by adipogenic and osteogenic induction media (R&D: following manufacturer’s protocols; data not shown). Fresh human CD34-positive cells were obtained by cell sorting from the mono-nucleated fraction of donor bone-marrow cells. Cells were cultured for seven days with stem cells factor (SCF) and thrombopoietin (TPO) for four days.

Preparation of soft and stiff hydrogel substrates

Adapted from [S4]. Glass cover slips (thickness #1.5, Fisher Scientific) were placed in boiling ethanol for 10 min, rinsed in distilled water (DW) and immersed in RCA (DW, hydrogen peroxidase (30%, Fisher Scientific), ammonium hydroxide (30%, Fisher Scientific) at 3:1:1 v/v) at 80 °C for 10 min and rinsed in DW. To remove water traces, glasses were rinsed in ethanol and then in chloroform and silanized in 0.1% allyltrichlorosilane (ATCS, Aldrich) in chloroform (Fisher Scientific) for 30 min. Silanized glasses were then
rinsed in chloroform, ethanol and DW and dried under vacuum. PA gel precursors were prepared by mixing acrylamide (AA, 40%, Sigma) and N,N'-methylenbisacrylamide (bis-AA 1.5% w/v in DW, Sigma) in PBS (Sigma). Gelation was initiated by adding 0.1% v/v tetramethylethylenediamine (TEMED, Sigma) and 0.1% w/v ammonium persulfate (Sigma) to gel precursor just before placing it at the center of the silanized cover slips and covering with RCA-treated glasses. Gels were allowed to polymerize while covalently binding the silanized glasses for 30-60 min. Non-silanized glasses were gently removed after immersing in PBS for 1-2 hours. Nominal gel elasticity was specified by varying acrylamide and cross-linker concentrations as calibrated by desktop rheometer. Gels were immersed in 10 mg/ml sulfo-SANPAH (Fisher Scientific) in 50 mM, pH 8.5 HEPES and reacted under 365 nm i-line exposure for 10 min. Collagen was mixed in 0.1 M acetic acid (Fisher Scientific) at equal volume and in 50mM, pH 8.5 HEPES to reach 0.2 mg/ml final concentration. Gels were immersed with collagen while agitated overnight at 37 °C. Prior to seeding cells, gels were UV-sterilized (cell culture hood UV light source) for three hours. During all preparation steps, gels were maintained in a hydrated state.

**siRNA and shRNA knockdown of MYH9, LMNA; myosin-IIA and kinase inhibitors**

All siRNAs used in this study were purchased from Dharmacon. Cells were passaged > 24 hours prior to transfection were and incubated with a complex of siRNA (30 nM; siLMNA: 5’-GGUGGUGACGACUGGGCU-3’; siMYH9: 5’-GGCCAAACCUGGCGAAUUU-3’ with complement sequence 5’-UUUAUUCGCCAGGUUUGGCCUU-3’ or scrambled siRNA siGENOME non-targeting siRNA #1 (Thermo Fisher Scientific)) and 1 µg/mL Lipofectamine 2000 according to the manufacturer’s instructions for 24 hours (in low glucose DMEM with 10% FBS). shLMNA construct (Sigma, TRCN0000061833: 5’-CGACTGGTGAGATTGACAAT-3’) was packed into a lentiviral delivery system, transduced into A549s by 1 µg/mL Polybrene for 72 hours and selected with 2 µg/mL puromycin. Racemic blebbistatin (EMD) was used at 30 µM (24 hr treatment). Kinase inhibitor RO3306 (Adipogen International) was used at 3.5 µM for no longer than 6 hours.
Transfection and transductions of lamin-A, myosin-IIA and respective phosphomimetic constructs

For MSCs: a construct expressing GFP-lamin-A under the EF1-alpha promoter [S5] was packed into a lentiviral delivery system. Cells were transduced at MOI 50 and evaluated for survival and proliferation (data not shown). The fraction of GFP-lamin-A positive cells prior to experiment was ~ 50%. GFP-positive cells were seeded on 6-well plates at very low density (less than one cell per 4X objective field of view). Colonies of GFP-lamin-A expressing MSCs were isolated using a cloning cylinder (Bel-Art Products, Pequannock, NJ), trypsinized and further expanded for experiments. Lamin-A constructs were transfected via electroporation following manufacturer’s protocols (MSCs kit, Nucleofector; Lonza); myosin-IIA constructs were transfected using Lipofectamine LTX with Plus reagent (Invitrogen), using 0.5 g DNA per well of a 6-well plate. For A549s: Transfections were performed with Lipofectamine LTX/Plus reagent (Invitrogen), as above. Transfection levels were similar across all constructs (within 20-30%) based on GFP intensities and densitometry of immunoblots (not shown). Phosphomutant constructs were packed into a lentiviral delivery system and transduced into lamin-A knockdown A549s. Transduction efficiencies ranged from 60-90%.

Plasmid constructs for WT GFP-myosin-IIA were obtained from Addgene. GFP-lamin-A and S1943D GFP-myosin-IIA plasmids were generous gifts from C. H. June (University of Pennsylvania, PA), D. M. Gilbert (State University of New York, Syracuse) and A. Bresnick (Albert Einstein College of Medicine, Bronx, NY). GFP-Lamin-A S22A, S22D and S22E plasmids were constructed by standard site directed mutagenesis (Stratagene).

Immunofluorescence imaging

Cells were fixed with 3.7% formaldehyde (Sigma-Aldrich) in PBS for 10 min at RT followed by PBS washing 2X for 5 min. Blocking and primary antibody staining was performed in 1% BSA in PBS. Primary antibody concentrations ranged between 1/300 – 1/500, depending on the stock concentration, and all primary antibodies were incubated at RT for 2 h or overnight at 4°C. All donkey secondary antibodies (Alexa Fluor dyes 488, 564, and 647) were stained for 1-2 hours at RT at 1:500 dilution in PBS and...
TRITC-phalloidin (Sigma-Aldrich) was used at a concentration of 100 ng/ml. Imaging for quantitative immunofluorescence of lamin-A, myosin-IIA and related phosphorylation-specific antibodies was performed using an inverted microscope (IX-71; Olympus) with either 20X (Olympus, NA-0.75) or 40X (NA-0.60) objectives, and a cooled CCD camera (Cascade; Photometrics) and image acquisition performed with Image Pro software (Media Cybernetics). Fixed cells were immuno- and histochemically-stained using the following antibodies and reagents: Myosin-IIA (mouse), monoclonal HSP90AB1 (Abcam); lamin-A,C pSer22, monoclonal cleaved lamin-A,C (Cell Signaling); polyclonal myosin-IIA, monoclonal lamin-A,C (mouse), polyclonal lamin-A,C (goat), polyclonal lamin-B1,2 (mouse), polyclonal cleaved lamin-A,C (Santa Cruz); polyclonal myosin-IIA, Phalloidin (Sigma). Fixed cells were mounted in mounting medium (Axell). To prevent volume distortions, samples prepared for confocal z-stacks quantification were not mounted. Prior to each experiment, lamp intensity and field of view homogeneity were calibrated pixel by pixel, relative to a fluorescent plastic standard. Image analysis was performed with in-house MatLab code that included background subtraction, cell and nuclear registration and intensity integration, morphological and statistical analyses.

**Confocal microscopy**

Laser scanning confocal fluorescence microscopy was carried out using the following systems: Leica Microsystems TCS SP8, 63×/ NA1.4 oil immersion objective (Fig. 2C) and Olympus Fluoview FV1000, 150X/NA1.45 or 60X/NA1.2 oil immersion objectives (Fig. 1A). Nuclear cross-sections orthogonal to the substrate were generated from 0.21 μm Z-stacks and constructed using Fiji [S6].

**Fluorescence recovery after photobleaching (FRAP)**

Confocal time-lapse imaging was acquired at 37 °C with 5% CO₂ in a humidified chamber with an inverted spinning-disk microscope (IX-81; Olympus) with a 14-bit high-resolution charge-coupled device (CCD) camera (HQ2; Photometrics) and MetaMorph software (Molecular Devices). Time lapse images were acquired with a 40X (Olympus, NA-0.75) and 60X water immersion lens (Olympus, NA-1.2), every 20 sec for 5-10 min after photobleaching. Cells were imaged in phenol-red free low glucose medium (DMEM, Life
Technologies) with 10% FBS and 1% penicillin/streptomycin. Image sequences were analyzed using MatLab custom designed code to quantify fluorescence recovery kinetics. After the first day cells were treated with 30 \( \mu \text{M} \) racemic blebbistatin (EMD; 24 hrs). Blebbistatin was washed out prior to FRAP in order to prevent light-induced toxicity.

**Immunoblotting**

Cells were trypsinized, pelleted and stored at -20 °C until analysis. Pellets were thawed and resuspended in 1X LDS lysis buffer supplemented with 1% protease and 1% phosphatase inhibitors and sonicated on ice (3 x 15 x 1 s pulses, intermediate power setting). After resting for 30 min on ice, samples were denatured at 80 °C with 0.5% \( \beta \)-mercaptoethanol v/v for 10 min. Samples were loaded onto bis-Tris 4-12% gradient gels for electrophoresis (100 V x 10 min; 160 V x 55 min) and then transferred (iBlot; Life Technologies: settings P3, 7 min) to blotting membrane. Band intensities were quantified using Fiji/ImageJ, relative to local background levels flanking the specific bands.

**Quantitative, label-free mass spectrometry**

SDS-PAGE gels (NuPAGE 4-12% Bis-Tris, Invitrogen) were run at 100 V for 10 min and 160 V for 25 min. Gel sections were washed (50% 0.2 M ammonium bicarbonate (AB) solution, 50% acetonitrile (ACN), 30 min at 37 °C), dried by lyophilization, incubated with a reducing agent (20 mM tris(2-carboxyethyl)phosphine (TCEP) in 25 mM AB solution at pH 8.0, 15 min at 37 °C) and alkylated (40 mM iodoacetamide (IAM) in 25 mM AB solution at pH 8.0, 30 min at 37 °C). The gel sections were dried by lyophilization before in-gel trypsinization (20 \( \mu \text{g/mL} \) sequencing grade modified trypsin in buffer as described in the manufacturer’s protocol (Promega), 18 hr at 37 °C with gentle shaking). The resulting solutions of tryptic peptides were acidified by addition of 50% digest dilution buffer (60 mM AM solution with 3% methanoic acid).

Peptide separations (5 \( \mu \text{L} \) injection volume) were performed on 15-cm PicoFrit column (75 \( \mu \text{m} \) inner diameter, New Objective) packed with Magic 5 \( \mu \text{m} \) C18 reversed-phase resin (Michrom
Bioresources) using a nanoflow high-pressure liquid chromatography system (Eksigent Technologies), which was coupled online to a hybrid LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) via a nanoelectrospray ion source. Chromatography was performed with Solvent A (Milli-Q water with 0.1% formic acid) and Solvent B (acetonitrile with 0.1% formic acid). Peptides were eluted at 200 nL/min for 3–28% B over 42 min, 28–50% B over 26 min, 50–80% B over 5 min, 80% B for 4.5 min before returning to 3% B over 0.5 min. To minimize sample carryover, a fast blank gradient was run between each sample. The LTQ-Orbitrap XL was operated in the data-dependent mode to automatically switch between full scan MS (m/z = 350-2000 in the Orbitrap analyzer (with resolution of 60,000 at m/z 400) and the fragmentation of the six most intense ions by collision-induced dissociation in the ion trap mass analyzer.

Raw mass spectroscopy data was processed using Elucidator (version 3.3, Rosetta Biosoftware). The software was set up to align peaks in data from samples derived from corresponding molecular weight regions of the 1D gels. Peptide and protein annotations were made using SEQUEST (version 28, Thermo Fisher Scientific) with full tryptic digestion and up to 2 missed cleavage sites. Peptide masses were selected between 800 and 4500 amu with peptide mass tolerance of 1.1 amu and fragment ion mass tolerance of 1.0 amu. Peptides were searched against a database compiled from UniRef100 (November 2010) mouse, plus contaminants and a reverse decoy database. A deltaCn of 0.01 and mass error limit of 20 ppm was used, resulting in a false positive rate of ~10%. In these experiments, only proteins detected with three or more peptides were considered. The peptide database was modified to search for alkylated cysteine residues (monoisotopic mass change, \( \Delta = +57.021 \) Da) and oxidized methionine (\( \Delta = +15.995 \) Da). In proteomic profiling experiments, we also considered the acetylation of lysine (\( \Delta = +42.011 \) Da), methylation of lysine and arginine (\( \Delta = +14.016 \) Da), and phosphorylation of serine, tyrosine, threonine, histidine and aspartate (\( \Delta = +79.966 \) Da). Ion currents of modified peptides were summed with their parent peptide. Peptides derived from trypsin or keratin were considered to be contaminants and were not used in subsequent calculations. When evaluating total ion current, only signals from annotated peptides were summed. The Peptide Ratio Fingerprinting algorithm was coded for Mathematica (version 8, Wolfram Research) and was used for all MS protein quantitation [S7].
Transcriptional profiling by DNA microarrays

Total RNA was extracted from cells using Trizol and purified by RNeasy (Qiagen) with on-column DNase digestion according to manufacturer’s protocol. Adherent cells were gently scraped in Trizol. Total RNA was amplified and converted to cDNA using WT-Ovation Pico kit (NuGen). Fragmented and biotin-labeled ST-cDNA was generated using WT-Ovation Exon Module (NuGen). Samples were tested with Human Gene 1.0 ST DNA microarrays (Affymetrix), used according to the manufacturer’s instructions. Expression data sets were analyzed by standard Robust Multi-array Averaging (RMA) methods.

Micropipette aspiration

Mesenchymal stem cells were harvested from culture, suspended in PBS + 1% BSA and treated in suspension with 0.5 µg/mL Latrunculin-A at 37 °C for 30 min. Cells were then washed with 1% BSA and re-suspended in 1% BSA supplemented with 0.2 µg/mL Latrunculin-A. A pulled glass micropipette was attached to a dual-stage water manometer with reservoirs of adjustable height. Suction was applied by syringe, and the corresponding pressure was measured by a pressure transducer (Validyne) calibrated with a mercury U-tube manometer. Pressures for different experiments were < 10 kPa. Images were acquired using a Nikon Eclipse TE300 inverted microscope using a 60X objective (Nikon, Plan Apo NA1.4) and a Cascade CCD camera (Roper Scientific). Images were captured every 30 sec over 5 min of aspiration at constant. Image were analyzed with ImageJ (NIH). Elastic stiffness was calculated according to previous work [S8]. Specifically, creep compliance \( J(L) = \frac{2\pi \cdot 2L}{3 \cdot R \cdot \Delta P} \) was computed per each nucleus as a function of nuclear extension \( L \) at each time point and then fitted by \( J(t) = A \cdot t^\alpha \). Nuclear relaxation times were computed with a threshold creep compliance overlapping with the dynamic range across conditions.

Systems Mechanobiology Gene Circuit (MGC) for coupled expression of lamin-A,C and myosin-IIA

Lamin-A,C \( (L) \) and myosin-IIA \( (M) \) message and protein circuitry is schematically presented in Figure 4F. In particular, expression kinetics of both are described by coupled rate equations for the respective transcripts (lower case) and proteins (upper case):
Matrix, lamin-A,C phosphorylation and actomyosin: supplemental materials

\[
\frac{dl}{dt} = \alpha_1 \cdot L - \beta_1 \cdot l
\]
Eq. 1

\[
\frac{dl}{dt} = \gamma_1 \cdot l - \delta_1 \cdot L^n_L \left( \frac{L^n_M}{K_L^{n_L} + L^n_L} \right)
\]
Eq. 2

\[
\frac{dm}{dt} = \alpha_2 \cdot M + \alpha_3 \cdot L - \beta_2 \cdot m
\]
Eq. 3

\[
\frac{dM}{dt} = \gamma_2 \cdot m - \delta_2 \cdot \left( \frac{M^n_M}{K_M^{n_M} + M^n_M} \right)
\]
Eq. 4

For simplicity, RNA degradation and translation are assumed linear in transcript concentration.

Lamin-A,C protein positively regulates one of its transcription factors, retinoic acid receptor (RAR), and myosin-IIA protein positively regulates one of its transcription factors, SRF, so that each enhances its own transcription (with rate constants \(\alpha_1, \alpha_2\)). In addition, lamin-A,C protein also enhances myosin-IIA transcription via the SRF pathway (rate constant \(\alpha_3\)). Our results indicate a mechanical regulation of protein phosphorylation, and so we describe lamin-A,C and myosin-IIA protein turnover with suitable Hill models (rate constants \(\delta_1, \delta_2\)). Specifically, lamin-A,C protein turnover is suppressed by myosin-generated stress with \(K_L = M^n_M\), while myosin-IIA protein turnover depends on matrix elasticity \(E\): \(K_M = \frac{E}{E + E_0}\).

A combined coordinate to describe nuclear tension and solutions to the MGC model

We sought to estimate a dimensionless measure of nuclear tension, \(\sigma\), in terms of the stiffness of the nucleus, \(E_{\text{nuc}}\), which depended on LMNA, and the nuclear strain, \(\epsilon\), which depended on changes in projected nuclear area, \(\Delta A\). In simplest form: \(\sigma = E_{\text{nuc}}\epsilon \sim \text{LMNA}^m\Delta A^n\), where \(m = 2.5\) conforms to scaling results for semi-flexible polymers [S9] as well as the enhanced nuclear viscosity with lamin-A,C [S10], and \(n = 0.5\) is consistent with the scaling of strain with changes in nuclear radius (Fig. 1F). When pSer22/LMNA was plotted against this nuclear tension coordinate using data from analyses of immuno-fluorescence images (MSCs cultured on soft vs. vs. thin/soft vs. stiff substrate, Fig. 1C; MSCs subjected to lamin-A,C KD or blebb treatment, Figs 1E; S1F; MSCs treated with kinase inhibitor, Figs. 3F, G; S3F, G), the data collapsed well onto a hyperbolic-like decay consistent with the MGC model (Figs. 1G, H; S3H).
Equations 1-4 of the MGC model were solved numerically at steady state (all derivatives = 0) with free parameters adjusted to collectively obtain the best agreement with experimental data (fitting was performed using Mathematica, Wolfram). The rate of protein turnover was modeled as being proportional to tension per molecule. Cooperative protein turnover was $n_M = 10.4$ and $n_L = 2$ with the half-maximum effect on myosin-IIA turnover occurring at $E_0 = 5 \text{kPa}$. As matrix stiffness and cell tension suppressed protein phosphorylation and turnover, steady state levels monotonically increased with matrix $E$, consistent with coupled mechano-regulation of lamin-A and myosin-IIA. Maximal agreement was achieved with the model's free parameters set to the following values: $\alpha_1 = 4.43$, $\alpha_2 = 10$, $\alpha_3 = 2$; $\beta_1 = 1.4$, $\beta_2 = 1.5$; $\gamma_1 = 0.071$, $\gamma_2 = 2.3$; $\delta_1 = 3$, $\delta_2 = 10.9$; $n_K = 0.08$, $n_M = 10.4$, $n_L = 2$ (Fig. 4G).
Supplemental references


