Concentration-Dependent Effects of Nuclear Lamins on Nuclear Size in Xenopus and Mammalian Cells

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*Running title: Nuclear lamin levels affect nuclear size

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Background: The nuclear lamina is a meshwork of intermediate lamin filaments lining the inner nuclear membrane.

Results: Altering lamin concentrations in Xenopus extracts, embryos, and cultured mammalian cells affects nuclear size.

Conclusion: Nuclear size is sensitive to lamin levels in Xenopus and mammalian cells.

Significance: Lamin expression patterns may contribute to changes in nuclear size during normal development and carcinogenesis.

ABSTRACT
A fundamental question in cell biology concerns the regulation of organelle size. While nuclear size is exquisitely controlled in different cell types, inappropriate nuclear enlargement is used to diagnose and stage cancer. Clarifying the functional significance of nuclear size necessitates an understanding of the mechanisms and proteins that control nuclear size. One structural component implicated in the regulation of nuclear morphology is the nuclear lamina, a meshwork of intermediate lamin filaments that lines the inner nuclear membrane. However, there has not been a systematic investigation of how the level and type of lamin expression influences nuclear size, in part due to difficulties in precisely controlling lamin expression levels in vivo. In this study, we circumvent this limitation by studying nuclei in Xenopus laevis egg and embryo extracts, open biochemical systems that allow for precise manipulation of lamin levels by the addition of recombinant proteins. We find that nuclear growth and size are sensitive to the levels of nuclear lamins, with low and high concentrations increasing and decreasing nuclear size, respectively. Interestingly, each type of lamin that we tested (lamins B1, B2, B3, and A) similarly affected nuclear size whether added alone or in combination, suggesting that total lamin concentration, and not lamin type, is more critical to determining nuclear size. Furthermore, we show that altering lamin levels in vivo, both in Xenopus embryos and mammalian tissue culture cells, also impacts nuclear size. These results have implications for normal development and carcinogenesis where both nuclear size and lamin expression levels change.

INTRODUCTION
Cell size varies greatly among different cell types and organisms, and especially during early development when cell division is rapid with little overall growth. How organelle size is appropriately regulated relative to cell size, a phenomenon referred to as scaling, is a fundamental yet unanswered question (1,2). The nucleus is one organelle that exhibits exquisite size scaling between different species and cell types, as well as during development and cell differentiation (3-6). Importantly, the normal scaling relationship between cell and nuclear size is often abrogated in cancers, and many cancers are diagnosed and staged based on graded increases in nuclear size (7,8). Whether altered nuclear size is a cause or consequence of cancer initiation and progression remains unknown. In order to understand the physiological significance of nuclear size, we must first understand the
proteins and mechanisms that contribute to the regulation of nuclear size. A major structural component of the nuclear envelope (NE) in metazoans is the nuclear lamina, a meshwork of intermediate lamin filaments that lines the inner nuclear membrane. Nuclear lamins are divided into two families, the A-type and B-type lamins. The overall structural organization of both lamin types includes a globular head domain at the amino-terminus, a central α-helical rod domain, and an immunoglobulin (Ig) domain and nuclear localization sequence (NLS) toward the carboxy-terminus, with A-type lamins having a unique C-terminal extension. A conserved CaaX box at the extreme carboxy-terminus undergoes farnesylation, targeting lamins to the inner nuclear membrane. While B-type lamins are fairly ubiquitously expressed, A-type lamin expression is generally restricted to differentiated cell types. Lamins play important roles in a wide range of nuclear functions, including chromatin organization, gene expression, DNA replication and repair, signal transduction, and mechanical properties of the nucleus (9,10). Lamin mutations give rise to a broad spectrum of diseases termed laminopathies. For instance, while the C-terminal farnesylation on lamin A (LA) is usually removed by normal processing events, mutations that cause retention of the farnesyl group underlie a premature aging disease called Hutchinson-Gilford progeria syndrome (11).

Lamins have previously been implicated in the regulation of nuclear morphology. For example, mouse embryonic fibroblasts depleted of lamin A/C exhibited misshapen nuclei and decreased nuclear stiffness, and cells deficient for lamin B1 (LB1) showed nuclear blebs and altered lamina meshwork size (12-14). In keratinocytes lacking all lamins, NE and endoplasmic reticulum (ER) membranes spread into the chromatin (15). Laminopathy patients generally exhibit irregularly shaped nuclei, including NE blebs and invaginations, further suggesting a role for lamins in the maintenance of normal nuclear morphology (16-18). However, nuclear size was not explicitly quantified in any studies that investigated how lamin expression in cells affects nuclear shape.

Studies in Xenopus have shown that certain lamin domains impact NE membrane structure. Addition of the C-terminal Ig-fold motif of lamin B3 (LB3) to nuclei pre-assembled in Xenopus egg extract inhibited nuclear expansion (19). Overexpression of lamins containing a CaaX motif in cultured Xenopus cells and oocytes induced proliferation of NE membrane and the formation of intranuclear membrane structures (20-22). In vitro nuclear assembly in Xenopus egg extract depleted of embryonic LB3 resulted in small nuclei that failed to expand normally (23,24). Comparing X. laevis and X. tropicalis egg extracts, faster rates of LB3 nuclear import in X. laevis correlated with faster nuclear expansion kinetics, and ectopic addition of LB3 to X. tropicalis egg extract increased the rate of nuclear growth (6). Similarly, LB3 overexpression in X. laevis embryos also resulted in increased nuclear size (5). While these studies implicated LB3 in nuclear size control, to date there has not been a systematic investigation of how the type and level of lamin expression influences nuclear size.

One limitation to investigating how lamin concentrations impact nuclear size is the difficulty in precisely controlling lamin expression levels in vivo. In this paper, we make use of Xenopus egg extracts, a powerful in vitro system for studying nuclear assembly and size (25,26). These extracts lack egg chromosomes but contain all the cytoplasmic proteins and membranes necessary to faithfully recapitulate nuclear assembly in vitro. Addition of demembranated Xenopus sperm to interphasic egg extract stimulates nuclear formation and expansion. Importantly, the open biochemical nature of this system offers many advantages over in vivo approaches. First, the composition of the extract can be easily and precisely manipulated by immunodepletion or addition of recombinant proteins at defined concentrations, as in the present study with lamin addition. Second, complex processes can be broken down into individual steps and studied in isolation, for instance here we specifically focus on nuclear expansion and size. Third, essential processes, that might pose viability issues in vivo, can be investigated in a cell-free setting. Furthermore, in this study we also utilize embryo extracts that offer all of the in vitro advantages of the egg extract system while allowing us to study endogenous embryonic nuclei in their native cytoplasm (26,27).

Studying nuclei assembled in Xenopus egg extracts, we find that nuclear growth and size are
sensitive to the levels of nuclear lamins, with low and high concentrations increasing and decreasing nuclear size, respectively. Interestingly, each type of lamin that we tested (LB1, LB2, LB3, and LA) similarly affected nuclear size, whether added alone or in combination. These data suggest that total lamin concentration, and not lamin type, is more critical to determining nuclear size. Furthermore, we show that altering lamin levels in vivo, both in *Xenopus* embryos and mammalian tissue culture cells, also impacts nuclear size. These results have implications for normal development and cell differentiation, as well as disease states like cancer, where both nuclear size and lamin expression often vary.

**EXPERIMENTAL PROCEDURES**

**Recombinant protein expression and purification:** The expression constructs for LB3 (pDL4), GFP-LB3 (pDL5), and GFP-LB3 R385P (pDL12) have already been described (6,28). To generate the CAAX box mutant, site-directed mutagenesis was performed on pDL5 to mutate the cysteine residues at amino acids 578 and 579 in LB3 to serines (pDL82) (29). Lamin A (DNASU Clone HsCD00296899) was cloned into pET30b at EcoRV and XhoI (pDL36), and GFP was cloned at BamHI and XhoI (pDL44). Lamin B1 (DNASU Clone HsCD00043675) was cloned into pET30b at BamHI and XhoI (pDL39), and GFP was cloned at BamHI and EcoRV (pDL45). Lamin B2 (Open Biosystems Clone 2961134) was cloned into pET30a at EcoRI and HindIII (pDL42), and GFP was cloned at BamHI and EcoRI (pDL53). The lamin proteins were expressed in bacteria and purified by immobilized-metal affinity chromatography, as previously described (6). Purified lamins were dialyzed into lamin storage buffer (10 mM HEPES, 300 mM KCl, 50 mM sucrose, 5% glycerol, pH 7.8).

*Xenopus laevis* egg and embryo extracts: Egg and embryo extracts were prepared as previously described (27). All *Xenopus* procedures and studies were conducted in compliance with the US Department of Health and Human Services Guide for the Care and Use of Laboratory Animals. Protocols were approved by the University of Wyoming Institutional Animal Care and Use Committee (Assurance #A-3216-01).

**Nuclear assembly and lamin addition:** Nuclear assembly reactions in *X. laevis* egg extract were assembled as previously described (27). After 30 – 35 minutes, when nuclear assembly was confirmed, 2.5 µl of recombinant lamin protein diluted in lamin storage buffer were added to 25 µl of extract containing pre-assembled nuclei. The concentration of the lamin stock solution was adjusted to vary the final concentration of lamin protein added to the reaction. For control reactions, 2.5 µl of lamin storage buffer were added. The reaction was allowed to proceed an additional 75 minutes at 20°C prior to fixation. We opted to supplement extract with recombinant lamins after nuclear assembly to ensure that ectopic lamin addition would not alter the kinetics of NE formation (data not shown).

**Embryo microinjections:** A plasmid consisting of pCS2+ containing GFP-LB3 (pDL19) was previously described (6). Lamin A (DNASU Clone HsCD00296899) was cloned into pCS107-GFP-3STOP (a gift from John Wallingford) at EcoRI and XhoI (pDL38). Lamin B1 (DNASU Clone HsCD00043675) was cloned into pCS107-GFP-3STOP at XhoI and EcoRV (pDL37). mCherry-LB2 was removed from pDL24 (see below) and cloned into pCS2+ at XbaI (pDL43). Plasmids were linearized with NotI (pDL19 and pDL43) or KpnI (pDL38 and pDL37), and mRNA was expressed from the SP6 promoter using the mMessage mMachine kit (Ambion). *X. laevis* embryos were prepared, microinjected with mRNA (ranging from 200 -1000 pg per embryo) or protein (see Figures for injected concentrations), cultured, and used to prepare embryo extracts, as previously described (5).

**Fixation and immunofluorescence:** Nuclei in egg or embryo extract were fixed, spun onto coverslips, and processed for immunofluorescence as previously described (27). For NPC staining, the primary antibody was mAb414 (Covance; 1:1000 dilution in PBS-3% BSA) and the secondary antibody was Alexa Fluor 568 anti-mouse IgG (Molecular Probes; 1:500 dilution in PBS-3% BSA). For GFP staining, the primary antibody was A-6455 (Invitrogen; 1:1000 dilution in PBS-3% BSA) and the secondary antibody was Alexa Fluor 488 anti-rabbit IgG (Molecular Probes; 1:1000 dilution in PBS-3% BSA). To confirm that centrifugation of nuclei onto coverslips was not altering apparent nuclear size, we quantified nuclei applied directly to a glass slide and visualized with Hoechst and GFP-NLS.
Similar to results reported in Figures 1 and 2, supplementing extract with 0.5 nM GFP-LB3, GFP-LB1, GFP-LB2, or GFP-LA increased cross-sectional nuclear area, while nuclei were smaller when lamins were added at 48 nM (data not shown).

**Microscopy and image quantification:** Nuclei were visualized with a fluorescence microscope (BX51; Olympus) using the following objectives: UPLFLN 20× (NA 0.50, air; Olympus), UPLFLN 40× (NA 0.75, air; Olympus), and UPLANAPO 60× (NA 1.20, water; Olympus). Images were acquired with a QIClick Digital charge-coupled device camera, mono, 12-bit (model QIClick-F-M-12) using cellSens software (Olympus). Images for measuring fluorescence staining intensity were acquired using the same exposure times. Total fluorescence intensity and cross-sectional nuclear area were measured from the original thresholded images using MetaMorph software (Molecular Devices). For publication, images were cropped and pseudocolored using ImageJ, but were otherwise unaltered. Where indicated, confocal imaging was performed on a spinning-disk confocal microscope based on an Olympus IX71 microscope stand equipped with a LMM/ILE/4 laser launch (Spectral Applied Research) and switchable two-fiber output to facilitate imaging through either a Yokogawa CSU-X1 spinning-disk head or TIRF illuminator. Confocal images were acquired with an ORCA-Flash4.0 V2 Digital CMOS C11440-22CU camera (ImagEM, Hamamatsu). Z-axis focus was controlled using a piezo Pi-Foc (Physik Instrumentes), and multiposition imaging was achieved using a motorized Ludl stage. An Olympus UPLSAPO 60x (N.A. 0.85, oil) objective was used. Image acquisition and analysis and all system components were controlled using Metamorph software. Z-slice thickness was generally 0.2 μm. NPC signal intensities were quantified from 4-6 20 μm² regions from a NE slice at the periphery of the nucleus to calculate NPC density. Area and circumference for each slice were measured, and total NE surface areas were calculated as the sum of the circumferences multiplied by the slice thickness. NPC density values were multiplied by the NE surface area values to calculate total NPC intensity per nucleus. NE areas for each slice were quantified from thresholded images and summed for each control nucleus and nucleus with crumpled morphology to estimate total membrane area for each nucleus.

**Western blots:** Known amounts of recombinant lamin proteins (see Figure Legends) and 10 μl each of stage 8, 11, 15, 19, and 28 embryo extracts were separated on 10% SDS-PAGE gels. Proteins were transferred to PVDF and probed with the following primary antibodies: anti-LB1 from rabbit at 1:1000 (Assay Biotechnology Company, R12-2224), anti-LB2 from rabbit at 1:1000 (Abgent, AP6737b), anti-LA from rabbit at 1:200 (Boster, PA1103). We verified that these lamin antibodies were unique for a given lamin type by testing them against our different recombinant lamin proteins. For normalization, a mouse antibody against Ran was used at 1:2000 (BD Transduction Laboratories, 610341). To measure the level of ectopic lamin expression in microinjected embryos, a rabbit antibody against GFP was used at 1:1000 (Invitrogen, A-6455). Secondary antibodies used at 1:20,000 were IRDye 800CW anti-rabbit (Li-Cor 926-32211) and IRDye 680RD anti-mouse (Li-Cor 925-68070). Blots were scanned on a Li-Cor Odyssey CLx instrument and band quantification was performed with ImageStudio.

**Tissue culture:** The control GFP-NLS plasmid was from Invitrogen (V821-20). Lamin B2 (Open Biosystems Clone 2961134) was cloned into pEmCherry-C2 (a derivative of pEGFP-C2, a gift from Anne Schlaitz) at BamHI and EcoRI (pDL24). GFP-lamin A was removed from pDL38 and cloned into pcDNA3.1(+) at BamHI and XhoI (pDL74). GFP-LB1 was removed from pDL37 and cloned into pcDNA3.1(+) at BamHI and EcoRV (pDL75). LB2 without a fluorescent tag was cloned from pDL24 into pcDNA3.1(+) at HindIII and XbaI (pDL78). LB1 without a fluorescent tag was cloned from pDL39 into pcDNA3.1(+) at BamHI and XhoI (pDL79). LA without a fluorescent tag was cloned from pDL36 into pcDNA3.1(+) at EcoRI and XhoI (pDL80). To knockdown expression of specific lamins, the following siRNAs were used: LB2 (Ambion, s39477), LA (Sigma, SASI_Hs02_00367643), LB1 (Sigma, SASI_Hs01_00203184), universal negative control (Sigma, SIC001). Cell lines used were MRC-5 normal human lung fibroblasts (a gift from Jason Gigley) and HeLa cells stably expressing GFP-histone H2B (a gift from Jay Gatlin). Both cell lines were cultured at 37°C with
5% CO₂ in DMEM media supplemented with 10% fetal bovine serum and 50 IU/mL penicillin and streptomycin. Cells were grown to confluency and seeded (1:6 for HeLa and 1:3 for MRC-5) in 24-well plates for both plasmid and siRNA transfections. At 70-90% confluency, transient plasmid transfections were performed with Lipofectamine 3000 (Invitrogen). Briefly, in one tube, 1.5 µg of DNA were added to 25 µL of Opti-MEM media plus 3 µL of P3000 reagent. In a separate tube, 1.5 µL of Lipofectamine 3000 reagent were added to 25 µL of Opti-MEM media. The two were mixed and incubated at room temperature for 5 minutes. Media was removed from the 24-well plates and replaced with 500 µL/well of fresh culture media and the 50 µL of DNA-lipofectamine complex. 6 hours (HeLa) or 48 hours (MRC-5) after transfection, cells were seeded onto acid-washed 18 mm square coverslips in 35 mm² dishes with 2 mL of fresh culture media. 24 hours later, coverslips were washed with PBS (without Mg²⁺ and Ca²⁺), fixed with 4% paraformaldehyde for 20 minutes, washed, stained with 5 µg/ml Hoechst for 5 minutes, washed, mounted in Vectashield (Vector Laboratories), and sealed with nail polish. Transient siRNA transfections were performed with Lipofectamine RNAiMAX (Invitrogen). To identify transfected cells, siRNAs were co-transfected with 1.5 µg of pEmCherry-C2. Briefly, 3 µL of 10 µM siRNA and 1.5 µg pEmCherry-C2 plasmid were diluted in 50 µL of Opti-MEM media, 3 µL of Lipofectamine RNAiMAX reagent were diluted in 50 µL of Opti-MEM, and the two were mixed and incubated at room temperature for 5 minutes. The 50 µL transfection mixture was added to 500 µL of culture media and incubated for 1 day (HeLa) or 3 days (MRC-5). Cells were then seeded onto coverslips, cultured an additional 24 hours, and fixed and processed as described above for plasmid transfections. Images were acquired and nuclear sizes quantified as described above under “Microscopy and image quantification.”

Statistics: Nuclear cross-sectional areas were measured from thresholded images in MetaMorph (Molecular Devices). For each coverslip, at least 50, and usually >800, nuclei were quantified, and areas were averaged. Unless otherwise noted, nuclear area measurements were normalized to controls. Averaging and statistical analysis were performed for independently repeated experiments. Two-tailed Student’s t-tests assuming equal variances were performed in Excel (Microsoft) to evaluate statistical significance. The p-values, number of independent experiments, and error bars are denoted in the Figure Legends.

RESULTS

Nuclear size is sensitive to the concentration of lamin B3 in X. laevis egg extract

Nuclear size decreases during early Xenopus development. After fertilization, the embryo undergoes 12 rapid cleavage cell divisions, giving rise to approximately 4000 cells at stage 8, also known as the midblastula transition (MBT). During this time, the average nuclear volume decreases ~3-fold (5). At the MBT, major zygotic transcription begins and cell divisions become longer and asynchronous. Gastrulation encompasses stages 10.5-12 (30), and average nuclear volume decreases another ~3-fold from the MBT up to this point in development (5). The major B-type lamin present in the egg and pre-MBT embryos is lamin B3 (LB3) (31). Previous work showed that LB3 levels increase in the embryo around the MBT, from 75 nM to 260 nM (6). We wanted to test if this increase in LB3 expression might contribute to the reduction in nuclear size around the MBT. For these experiments, we used bacterially-expressed and purified GFP-LB3. Previous studies have shown that recombinant lamins exhibit structures and behaviors similar to endogenous lamins, existing as dimers/tetramers or paracrystals depending on buffer conditions (32-41), and being chaperoned by importins (42). In addition to our bacterially-expressed GFP-LB3 being imported into nuclei and incorporated into the NE (6), we also determined that it becomes farnesylated in X. laevis egg extract (data not shown), further justifying the use of these recombinant lamin proteins in our study.

We assembled nuclei in X. laevis egg extract and then supplemented the extract with increasing concentrations of recombinant GFP-LB3. Nuclear expansion was allowed to proceed for an additional 75 minutes, and we then compared nuclear size to control extracts to which buffer was added in place of recombinant LB3 protein. Since nuclei expand continuously in egg extract, this time point serves as a proxy for the nuclear expansion rate, and at this time nuclei...
have nearly reached their largest size before physical integrity of the NE becomes compromised (6). Low levels of additional LB3, less than 10 nM, resulted in larger nuclei with 10-40% greater surface areas (Fig. 1). While incorporation of GFP-LB3 into the NE was barely visible at sub-nanomolar concentrations, nuclear GFP-LB3 was evident at higher concentrations (Fig. 1A). Furthermore, immunofluorescence against GFP revealed NE rim localization of GFP-LB3 at lower concentrations, demonstrating the recombinant protein correctly localizes and is functional (Fig. 1B). Addition of GFP-LB3 mutant proteins defective for farnesylation or assembly into the lamina failed to increase nuclear size (Fig. 1D). Large nuclei had approximately the same total NPC staining as control nuclei, and bulk import monitored with GFP-NLS was not significantly altered by lamin addition (data not shown).

Higher LB3 levels greater than 10 nM generally slowed nuclear expansion, resulting in nuclei that were smaller than the control. Concomitant with this reduction in nuclear size, we often noted intranuclear GFP-LB3 puncta and a more irregular crumpled NE morphology (~25% of nuclei), whereas the GFP-LB3 was almost exclusively NE-localized at lower concentrations. Based on confocal imaging, we determined that the total NE surface area of crumpled nuclei was less than that of control nuclei (data not shown).

To confirm that the GFP tag was not contributing to the observed effects on nuclear size, we tested a LB3 protein without a GFP tag and obtained similar results (Fig. 1C-D). Previous work showed that the addition of other nuclear targeted proteins like GFP-NLS and nucleoplasmin across a wide range of concentrations had no effect on nuclear size (6). Our experiments with GFP-NLS were consistent, as adding GFP-NLS to nuclei assembled in egg extract did not affect nuclear size (data not shown), supporting the idea that the effects we see here are lamin-specific. The amount of LB3 that resulted in larger or smaller nuclei varied between experiments, consistent with previously noted extract-to-extract variability (6,27,43). Nonetheless, a general trend was evident where low levels of LB3 increased nuclear size and higher levels of LB3 decreased nuclear size.

Nuclear size is sensitive to the concentrations of somatic A- and B-type lamins in X. laevis egg extract

Having observed a concentration-dependent effect of LB3 on nuclear size, we next tested the effects of different lamin types on nuclear size. We generated recombinant GFP-lamin B1 (GFP-LB1), GFP-lamin B2 (GFP-LB2), and GFP-lamin A (GFP-LA) proteins and verified their proper localization to nuclei assembled in X. laevis egg extract (Fig. 2A). We next titrated different levels of each lamin into egg extract containing pre-assembled nuclei and quantified their effect on nuclear size. Each individual lamin affected nuclear size similarly to LB3. Low levels modestly increased nuclear size, while higher levels reduced nuclear growth leading to nuclei that were smaller than control nuclei (Fig. 2). Similar results were obtained with lamin proteins lacking a GFP tag (Fig. 2B, open symbols). We verified by intensity of Hoechst staining that the DNA content of larger and smaller nuclei did not differ significantly from that of control nuclei (data not shown). In general, the threshold lamin concentration where nuclear size switched from larger to smaller occurred ~10 nM. Interestingly, at high lamin concentrations greater than ~100 nM, nuclear size was similar to or even slightly larger than control nuclei in some experiments (Fig. 2B).

Mimicking embryonic lamin levels in X. laevis egg extract

We next wanted to compare our egg extract results to the in vivo lamin levels present during normal X. laevis development. It was previously reported that LB1 is first expressed around the MBT, followed by LB2 at stage 12, while LB3 levels drop after stage 20 (31,44), roughly consistent with recent transcriptomics and proteomics studies (45-47). To quantitatively assess lamin expression levels over the course of early Xenopus development, we performed immunoblots using different stage embryo extracts. We first tested commercial antibodies against our recombinant lamin proteins to verify that each antibody was specific for a single lamin type (data not shown). We then immunoblotted different stage embryo extracts along with known amounts of recombinant lamin proteins, and estimated the amount of each lamin type present at different stages of embryonic development (Fig. 3A).
With this quantitative information in hand, we added different lamin concentrations to nuclei assembled in egg extract to approximate the endogenous lamin concentrations found in stage 8, 11, and 19 embryos. We found that lamin combinations ranging from 45-225 nM in total slowed nuclear expansion and resulted in nuclei that were smaller than buffer-treated controls (Fig. 3B). This effect was similar to what we observed when we supplemented extract with single lamins (Figs. 1-2), as well as when we added lamins to extract immunodepleted of LB3 (data not shown). In all cases, similar concentration-dependent effects were observed where low levels of single lamins led to larger nuclei, while higher levels of single lamins or lamin combinations greater than ~10 nM resulted in smaller nuclei relative to controls. Lamin combinations did not appear to have any additive effects, and single lamins added at the same total concentrations caused similar changes in nuclear size. Taken together, these data suggest that in terms of nuclear size regulation, lamin concentration is more important than the specific type of lamin expressed, with potential redundancy across lamin types.

**Lamin expression levels impact in vivo nuclear size in Xenopus embryos**

To test how lamin expression modulates nuclear size in vivo, we examined how altering lamin levels in *Xenopus* embryos affected nuclear size. First, to segue between the in vitro and in vivo experiments, we generated pre-MBT early stage 8 embryo extracts containing endogenous embryonic nuclei and supplemented these extracts with defined concentrations of recombinant lamins. After a 60-minute incubation, we quantified changes in nuclear size. Low concentrations of added recombinant lamins, generally less than ~10 nM, caused nuclear surface area to expand by 20-50% relative to control nuclei supplemented with only lamin storage buffer. Higher lamin concentrations led to more modest increases or no change in nuclear size (Fig. 4). Notably, nuclei did not shrink smaller when embryo extract was supplemented with high lamin concentrations. Similar to results in egg extract, in spite of extract-to-extract variability, each lamin type showed a similar general effect on nuclear size, and intranuclear lamin aggregates were apparent at high lamin concentrations (Fig. 4A).

Moving into the in vivo system, we microinjected one-cell stage *X. laevis* embryos with recombinant lamin proteins and allowed the embryos to develop to early stage 8. We then isolated and quantified endogenous embryonic nuclei. Nuclei in embryos injected with LB1, LB2, LB3, or LA to a final concentration of 1 nM were larger compared to buffer injected control embryos. Embryos injected with higher concentrations of any single lamin or combinations of lamins exhibited nuclear sizes similar to or slightly smaller than control embryos (Fig. 5A). Due to the difficulty in knowing the lifetime and fate of these microinjected proteins, we next microinjected mRNAs that would allow for more long-term lamin expression.

To determine if ectopic lamin expression within the embryo would alter nuclear size in later stages of development, we microinjected single-cell embryos with mRNA encoding the different lamin types, allowed embryos to develop to stage 11-12, and then isolated and quantified nuclei from these embryos. First, we showed that the fluorescently labeled lamins encoded by the injected mRNAs were expressed, both by immunoblot (data not shown) and by their functional incorporation into the nucleus (Fig. 5B). Next, we quantified nuclear size as a function of the level of ectopic lamin protein expression, estimated from immunoblots. Consistent with our in vitro results, lower lamin expression levels led to increased nuclear size in embryos and greater lamin expression decreased nuclear size compared to control-injected embryos (Fig. 5C). The approximate concentration of ectopic lamin expression where nuclear size shifted from being larger to smaller occurred ~150-200 nM. This concentration is an order of magnitude greater than the 10 nM threshold observed for the in vitro studies with egg and pre-MBT embryo extracts. While these different thresholds may result from differences between the in vitro and in vivo systems, a more likely explanation is that lamin concentrations affect nuclear size differently depending on the developmental context (i.e. egg or early embryo versus gastrula). This idea is further explored in the Discussion. Importantly, embryos expressing high levels of ectopic lamins do not undergo developmental arrest, are viable, and can develop into tadpoles (data not shown). Taken together, these data demonstrate that in vivo...
embryonic nuclear size is sensitive to lamin expression levels.

**Lamin expression levels impact in vivo nuclear size in mammalian cells**

To extend our in vivo *Xenopus* results, we tested if altering lamin expression levels in mammalian tissue culture cells also affected nuclear size. We transiently transfected HeLa and MRC-5 (normal human lung fibroblast) cells with different lamin expression constructs and verified that the ectopically expressed fluorescently-labeled lamins were localized to the nucleus (Fig. 6A, C). Generally, the ectopic lamin protein was properly localized to the nucleus in a smooth ring within the NE and an oval nuclear morphology was maintained. Nuclear size was quantified 1-3 days after plasmid transfection. In both cell types, ectopic expression of LB1, LB2, or LA resulted in increased average nuclear size compared to GFP-NLS control-transfected cells. Lamin expression increased nuclear surface area by 5-25% in HeLa cells and 10-50% in MRC-5 cells (Fig. 6B, D). Nuclear size was similarly increased by ectopic LB2 expression in U2OS osteosarcoma cells (data not shown). HeLa cells transfected with lamin expression constructs lacking fluorescent tags exhibited similar increases in nuclear size, confirming that the tags were not contributing to observed effects on nuclear size (Fig. 6D). The level of ectopic lamin expression over endogenous levels in HeLa cells, as measured by immunoblotting, was 49% ± 19% for LB1, 45% ± 29% for LB2, and 17% ± 6% for LA (average ± SD, n=3 experiments; Fig. 6D). When examined on a cell-by-cell basis, a weak positive correlation was noted between the signal intensity of the fluorescently-labeled lamins and nuclear size, in both transfected HeLa and MRC-5 cells (data not shown). While it is difficult to precisely control lamin expression levels by this approach, we noted a small proportion of transfected cells that expressed particularly high levels of the fluorescently-labeled lamins. In these cases, the nuclei tended to be abnormally shaped and smaller than controls, the NE was wrinkled, and there were intranuclear lamin puncta that excluded chromatin and appeared to disrupt chromatin organization. Cytoplasmic lamin puncta were also observed in some cells.

In a complementary set of experiments, we observed that nuclear size was reduced in HeLa cells transiently transfected with siRNA to individually knockdown the levels of different lamin types (Fig. 6E, F). By DNA staining and histone labeling, the chromatin in these small nuclei appeared highly condensed compared to cells transfected with a control siRNA. We confirmed that these cells were not undergoing apoptosis by absence of staining for activated caspase-3/7 (data not shown). We verified by intensity of Hoechst staining that nuclear DNA content did not significantly differ in cells ectopically expressing lamins or in siRNA treated cells (data not shown). Lamin overexpression or depletion did not appear to induce cell cycle arrest, as there were no obvious changes in cell growth rates or mitotic indices. Furthermore, transfected HeLa cells arrested in G2/M still exhibited increased or decreased nuclear size upon ectopic lamin expression or lamin depletion, respectively (data not shown). Taken together, these data show that mammalian nuclear size is also sensitive to lamin expression levels.

**DISCUSSION**

In this study, we report that the sizes of nuclei assembled in *X. laevis* egg extract are sensitive to the nuclear lamin concentration. Furthermore, altering lamin levels in embryos and mammalian tissue culture cells also leads to changes in nuclear size. We observed a trend indicating that total lamin concentration is more relevant to dictating nuclear size than the type of lamin expressed. Our data also suggest that cellular context matters in determining how lamin concentrations will affect nuclear size. In the egg and early pre-MBT embryo, very low levels of lamin addition lead to nuclear size increases while higher lamin levels decrease nuclear size. In later stage, post-MBT embryos, the curve is shifted so that relatively high lamin levels are necessary to increase nuclear size and even higher levels decrease nuclear size (Fig. 7). Therefore, when examining the impact of lamins on nuclear size, it is important to consider both lamin expression levels as well as the developmental stage and cell type. A recent study showed that lamin levels are critical in regulating lamina assembly and the association of other NE proteins such as nuclear pore complexes and emerin (48). It seems likely that lamin
concentrations might impact other known functions and properties of the lamina, for instance in regulating chromatin organization, DNA replication and repair, gene expression, aging and cellular senescence, and biomechanical properties of the nucleus (9,11,49).

How might lamins lead to larger nuclear size? It is well established that lamins are required for nuclear expansion during interphase, for example nuclei assemble but fail to grow in *X. laevis* egg extracts depleted of lamins (23,24). The NE is continuous with the ER and can be thought of as an extended ER sheet, and it has been proposed that there is a tug-of-war relationship between the two membrane systems (50). During NE assembly, ER tubules contact and spread across the chromatin (51), and altering the relative proportions of ER tubules and sheets can have concomitant effects on nuclear size (5,50). As nuclei expand and import lamins during interphase, the mechanical properties of the assembling lamina meshwork may resist the tendency of ER tubules to extract membrane from the NE. Greater lamin import might therefore be expected to result in increased NE growth. Consistent with this idea, the nuclear lamina has been shown to be an important determinant of NE stiffness (13,52).

Somewhat surprising was the relatively low amounts of ectopic lamins sufficient to increase nuclear size. The import capacity of *X. laevis* egg extract is extremely high and even small increases in import kinetics can have a dominant negative effect on nuclear size (6). Furthermore, we have determined that all LB3 present in a stage 8 embryo (~4000 cells) is incorporated into the nuclei, with little LB3 remaining in the cytoplasm (data not shown), so we expect that most of the endogenous LB3 is incorporated into the in vitro assembled nuclei. Thus, it seems reasonable that a relatively small influx of ectopic lamins can lead to an increase in nuclear size, given the extremely fast import kinetics of the egg extract and the fact that the endogenous lamins are already incorporated into nuclei.

Why might higher lamin levels lead to decreased nuclear size? One possibility is that when lamins are incorporated into the lamina above a certain threshold amount, the mechanical properties of the lamina and NE change such that the balance between ER and nuclear membranes shifts toward the ER. As previously discussed, when ER tubules are able to extract membrane from the NE, this leads to a corresponding reduction in nuclear size. In this scenario, the increased total lamin levels observed in *X. laevis* embryos around the MBT may contribute to developmental scaling of nuclear size. It will be interesting to investigate how the stiffness and dynamics of the nuclear lamina change during developmental progression and as a function of the amount of lamin protein incorporated. In this study, we noted that the addition of high lamin concentrations to egg extract nuclei, as well as high lamin expression in cells, led to misshapen wrinkled nuclei, suggesting altered nuclear mechanics.

It was previously reported that high rates of nuclear import correlate with the appearance of intranuclear lamin aggregates and puncta (6), providing another possible explanation for why high lamin levels decrease nuclear size. Perhaps when lamins are imported into the nucleus too rapidly, they are unable to properly assemble into the nuclear lamina meshwork. Instead, unincorporated lamins might aggregate within the nucleus, and these aggregates may even extract lamins already assembled into the lamina, thus leading to smaller nuclei. We observed such intranuclear puncta when high lamin levels were added to egg and embryo extracts, as well as in a small number of transfected mammalian cells that expressed the highest levels of ectopic lamins, consistent with work in other cultured cell systems where lamin overexpression generated membrane-containing aggregates within nuclei (20-22). In support of this hypothesis, high concentrations of a LB3 CAAX box mutant defective for farnesylation still formed intranuclear puncta and decreased nuclear size, while a LB3 point mutant defective for lamina assembly did not (Fig. 1D). This effect might be similar to previous reports of nuclear lamina disassembly by the expression of dominant negative lamin mutants (41,53), and is consistent with precipitation of recombinant lamins in lamin storage buffer at concentrations higher than ~1 mg/ml (data not shown).

Our data underscore that lamin concentration-dependent effects on nuclear size differ depending on the developmental stage. In particular, high lamin levels that cause nuclei to become smaller in early stages of development
lead to nuclear size increases in post-MBT embryos. One possible explanation is that endogenous lamin levels are lower in post-MBT embryos compared to pre-MBT embryos. However, when we correct for total lamin concentration, we still find that the nuclear size data from different developmental stages do not overlap (Fig. 7). Instead, we propose that the onset of zygotic transcription and cell differentiation that occur at the MBT induces a substantial change in cellular context that might explain why the lamin concentration effect on nuclear size differs in post-MBT embryos. Nuclear import capacity is high in the early embryo, so even relatively low additional lamin amounts (>10 nM) might reduce nuclear size due to rapid import. The reduced nuclear import capacity in post-MBT embryos may allow nuclei to accommodate higher lamin levels without forming aggregates (6). In this way, lamin concentrations that interfere with nuclear growth in the egg and early embryo are tolerated at later stages of development and result in increased nuclear size. Similarly, relative import capacities in somatic and cancer cells may be an important determinant of how lamin expression levels impact nuclear size (54).

We recently reported that conventional protein kinase C (PKC) activity is sufficient to cause nuclear shrinking with a concomitant removal of lamins from the NE (27), and nuclear lamins are known substrates for PKC during both mitosis and interphase (55-57). In addition, nuclear PKC activity and localization increase during developmental progression past the MBT, contributing to developmental nuclear scaling (27). Interestingly, in the present study, addition of high lamin levels to endogenous nuclei in pre-MBT embryonic extract did not cause these nuclei to shrink smaller. Changes in PKC activity during development may offer another explanation for why different lamin concentration regimes affect nuclear size differently in early and late stage embryos. Low PKC activity early in development might correspond to slow lamina dynamics and reduced capacity to appropriately assemble high lamin amounts into the lamina. Conversely, late stage embryos may be able to accommodate higher lamin levels due to greater PKC activity and increased lamina dynamics.

Our study of how lamin levels affect nuclear size has potential implications in normal development and disease. First, our data suggest that changes in total lamin levels during *Xenopus* embryogenesis may represent one mechanism that contributes to developmental reductions in nuclear size. Second, nuclear size is usually increased in cancer cells, and altered nuclear morphology is a key diagnostic and prognostic feature used by pathologists (7,8). The changes in nuclear size that we report here fall within the clinically relevant range of nuclear size changes in cancer. For instance, nuclear area increases 29% from stage 1 to stage 4 breast cancer (58), 16% from primary to metastatic melanoma (59), and 39% from benign hyperplasia to prostate carcinoma (60). Furthermore, we recently reported that increasing nuclear surface area in *Xenopus* embryos by 36% was sufficient to change the timing of the MBT (5), demonstrating that the changes in nuclear size we report in the current study are likely also physiologically relevant in the context of vertebrate development.

Both increased and decreased lamin protein expression have been reported in a variety of different cancers (61,62), making it difficult to understand how lamin levels might contribute to nuclear size changes in cancer. LB1 protein expression is 4.4- and 7.2-fold increased in early and late stage liver cancer, respectively (63,64), and 1.7-fold increased in colorectal carcinoma (65). A general increase in expression of LB1 and LB2 was noted in squamous cell carcinoma and prostate cancer (66,67). On the other hand, a reduction in LB1 expression was observed in colon and gastric cancers (68), and many small cell lung cancers are negative for B-type lamins (69,70). From benign to low grade prostate cancer, lamin A protein levels decrease 1.5-fold, and then increase 2.1-fold from low grade to high grade (71). Gastric carcinomas, fibrosarcomas, colorectal carcinomas, and small cell lung cancers exhibit 20%, 50%, 60%, and 80% reductions in lamin A/C levels, respectively (70,72-74). Loss or absence of lamin A/C expression was noted in basal and squamous cell carcinomas, primary colon carcinomas and adenomas, gastric cancer, small cell lung cancer, leukemia, and lymphomas (66,68-70,75,76). Conversely, lamin A/C, LB1, and LB2 are expressed at 2- to 4-fold higher levels in high-grade ovarian tumors (77-79). Our data offer one possible resolution to these apparently contradictory observations, namely that either

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increased or decreased lamin levels can lead to increased nuclear size in cancer depending on the cellular context. How changes in lamin levels might impact nuclear size will depend on the lamin complement in the cell type of origin and cancer-associated changes in nuclear transport and other nuclear structural components. The new mechanistic data presented here about the role of lamins in nuclear size regulation will facilitate future studies into the functional significance of nuclear size.

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Author contributions: PJ, LJE, and DLL designed the study and wrote the paper. DLL constructed plasmids, synthesized mRNA for microinjections, and purified recombinant proteins. PJ performed egg extract, embryo extract, and embryo microinjection experiments. LJE performed tissue culture experiments. XL performed embryo microinjection experiments. TN and PC performed egg extract experiments. All authors analyzed the results and approved the final version of the manuscript.

REFERENCES
1. Levy, D. L., and Heald, R. (2012) Mechanisms of intracellular scaling. *Annu Rev Cell Dev Biol* **28**, 113-135
2. Chan, Y. H., and Marshall, W. F. (2010) Scaling properties of cell and organelle size. *Organogenesis* **6**, 88-96
3. Conklin, E. (1912) Cell size and nuclear size. *J. Exp. Embryol*. **12**, 1-98
4. Wilson, E. B. (1925) The karyoplasmic ratio. in *The Cell in Development and Heredity*, Third Ed., The Macmillan Company, New York. pp 727-733
5. Jevtic, P., and Levy, D. L. (2015) Nuclear size scaling during Xenopus early development contributes to midblastula transition timing. *Curr Biol* **25**, 45-52
6. Levy, D. L., and Heald, R. (2010) Nuclear size is regulated by importin alpha and Ntf2 in Xenopus. *Cell* **143**, 288-298
7. Zink, D., Fischer, A. H., and Nickerson, J. A. (2004) Nuclear structure in cancer cells. *Nat Rev Cancer* **4**, 677-687
8. Jevtic, P., and Levy, D. L. (2014) Mechanisms of nuclear size regulation in model systems and cancer. *Adv Exp Med Biol* **773**, 537-569
9. Dittmer, T. A., and Misteli, T. (2011) The lamin protein family. *Genome Biol* **12**, 222
10. Wilson, K. L., and Berk, J. M. (2010) The nuclear envelope at a glance. *J Cell Sci* **123**, 1973-1978
11. Davidson, P. M., and Lammerding, J. (2014) Broken nuclei--lamins, nuclear mechanics, and disease. *Trends Cell Biol* **24**, 247-256
12. Lammerding, J., Schulze, P. C., Takahashi, T., Kozlov, S., Sullivan, T., Kamm, R. D., Stewart, C. L., and Lee, R. T. (2004) Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *J Clin Invest* **113**, 370-378
13. Lammerding, J., Fong, L. G., Ji, J. Y., Reue, K., Stewart, C. L., Young, S. G., and Lee, R. T. (2006) Lamins A and C but not lamin B1 regulate nuclear mechanics. *J Biol Chem* **281**, 25768-25780
14. Shimi, T., Pfleghaar, K., Kojima, S., Pack, C. G., Solovei, I., Goldman, A. E., Adam, S. A., Shumaker, D. K., Kinjo, M., Cremer, T., and Goldman, R. D. (2008) The A- and B-type nuclear lamin networks: microdomains involved in chromatin organization and transcription. *Genes Dev* **22**, 3409-3421

15. Jung, H. J., Tatar, A., Tu, Y., Nobumori, C., Yang, S. H., Goulbourne, C. N., Herrmann, H., Fong, L. G., and Young, S. G. (2014) An absence of nuclear lamins in keratinocytes leads to ichthyosis, defective epidermal barrier function, and intrusion of nuclear membranes and endoplasmic reticulum into the nuclear chromatin. *Mol Cell Biol* **34**, 4534-4544

16. Paradisi, M., McClintock, D., Boguslavsky, R. L., Pedicelli, C., Worman, H. J., and Djabali, K. (2005) Dermal fibroblasts in Hutchinson-Gilford progeria syndrome with the lamin A G608G mutation have dysmorphic nuclei and are hypersensitive to heat stress. *BMC Cell Biol* **6**, 27

17. Capell, B. C., Erdos, M. R., Madigan, J. P., Fiordalisi, J. J., Varga, R., Conneely, K. N., Gordon, L. B., Der, C. J., Cox, A. D., and Collins, F. S. (2005) Inhibiting farnesylation of progerin prevents the characteristic nuclear blebbing of Hutchinson-Gilford progeria syndrome. *Proc Natl Acad Sci U S A* **102**, 12879-12884

18. Mallampalli, M. P., Huyer, G., Bendale, P., Gelb, M. H., and Michaelis, S. (2005) Inhibiting farnesylation reverses the nuclear morphology defect in a HeLa cell model for Hutchinson-Gilford progeria syndrome. *Proc Natl Acad Sci U S A* **102**, 14416-14421

19. Shumaker, D. K., Lopez-Soler, R. I., Adam, S. A., Herrmann, H., Moir, R. D., Spann, T. P., and Goldman, R. D. (2005) Functions and dysfunctions of the nuclear lamin Ig-fold domain in nuclear assembly, growth, and Emery-Dreifuss muscular dystrophy. *Proc Natl Acad Sci U S A* **102**, 15494-15499

20. Prufert, K., Vogel, A., and Krohne, G. (2004) The lamin CxxM motif promotes nuclear membrane growth. *J Cell Sci* **117**, 6105-6116

21. Prufert, K., Alsheimer, M., Benavente, R., and Krohne, G. (2005) The myristoylation site of meiotic lamin C2 promotes local nuclear membrane growth and the formation of intranuclear membranes in somatic cultured cells. *Eur J Cell Biol* **84**, 637-646

22. Ralle, T., Grund, C., Franke, W. W., and Stick, R. (2004) Intranuclear membrane structure formations by CaaX-containing nuclear proteins. *J Cell Sci* **117**, 6095-6104

23. Newport, J. W., Wilson, K. L., and Dunphy, W. G. (1990) A lamin-independent pathway for nuclear envelope assembly. *J Cell Biol* **111**, 2247-2259

24. Jenkins, H., Holman, T., Lyon, C., Lane, B., Stick, R., and Hutchison, C. (1993) Nuclei that lack a lamina accumulate karyophilic proteins and assemble a nuclear matrix. *J Cell Sci* **106 (Pt 1)**, 275-285

25. Chan, R. C., and Forbes, D. I. (2006) In vitro study of nuclear assembly and nuclear import using Xenopus egg extracts. *Methods Mol Biol* **322**, 289-300

26. Edens, L. J., and Levy, D. L. (2014) Size scaling of subcellular organelles and structures in Xenopus laevis and Xenopus tropicalis. in *Xenopus Development* (Kloc, M., and Kubiak, J. Z. eds.), First Edition Ed., John Wiley & Sons, Inc., Hoboken, New Jersey. pp 325-345

27. Edens, L. J., and Levy, D. L. (2014) cPKC regulates interphase nuclear size during Xenopus development. *J Cell Biol* **206**, 473-483

28. Heald, R., and McKeon, F. (1990) Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell* **61**, 579-589

29. Kitten, G. T., and Nigg, E. A. (1991) The CaaX motif is required for isoprenylation, carboxyl methylation, and nuclear membrane association of lamin B2. *J Cell Biol* **113**, 13-23

30. Nieuwkoop, P. D., and Faber, J. (1967) *Normal Table of Xenopus laevis (Daudin)*, 2nd ed., North-Holland Publishing Company, Amsterdam

31. Stick, R., and Hausen, P. (1985) Changes in the nuclear lamina composition during early development of Xenopus laevis. *Cell* **41**, 191-200
32. Heitlinger, E., Peter, M., Haner, M., Lustig, A., Aebi, U., and Nigg, E. A. (1991) Expression of chicken lamin B2 in Escherichia coli: characterization of its structure, assembly, and molecular interactions. *J Cell Biol* **113**, 485-495
33. Gu, L., Troncoso, J. C., Wade, J. B., and Monteiro, M. J. (2004) In vitro assembly properties of mutant and chimeric intermediate filament proteins: insight into the function of sequences in the rod and end domains of IF. *Exp Cell Res* **298**, 249-261
34. Karabinos, A., Schunemann, J., Meyer, M., Aebi, U., and Weber, K. (2003) The single nuclear lamin of Caenorhabditis elegans forms in vitro stable intermediate filaments and paracrystals with a reduced axial periodicity. *J Mol Biol* **325**, 241-247
35. Foeger, N., Wiesel, N., Lotsch, D., Mucke, N., Kreplak, L., Aebi, U., Gruenbaum, Y., and Herrmann, H. (2006) Solubility properties and specific assembly pathways of the B-type lamin from Caenorhabditis elegans. *J Struct Biol* **155**, 340-350
36. Isobe, K., Gohara, R., Ueda, T., Takasaki, Y., and Ando, S. (2007) The last twenty residues in the head domain of mouse lamin A contain important structural elements for formation of head-to-tail polymers in vitro. *Biosci Biotechnol Biochem* **71**, 1252-1259
37. Heitlinger, E., Peter, M., Lustig, A., Villiger, W., Nigg, E. A., and Aebi, U. (1992) The role of the head and tail domain in lamin structure and assembly: analysis of bacterially expressed chicken lamin A and truncated B2 lamins. *J Struct Biol* **108**, 74-89
38. Gieffers, C., and Krohne, G. (1991) In vitro reconstitution of recombinant lamin A and a lamin A mutant lacking the carboxy-terminal tail. *Eur J Cell Biol* **55**, 191-199
39. Schirmer, E. C., Guan, T., and Gerace, L. (2001) Involvement of the lamin rod domain in heterotypic lamin interactions important for nuclear organization. *J Cell Biol* **153**, 479-489
40. Schirmer, E. C., and Gerace, L. (2004) The stability of the nuclear lamina polymer changes with the composition of lamin subtypes according to their individual binding strengths. *J Biol Chem* **279**, 42811-42817
41. Ellis, D. J., Jenkins, H., Whitfield, W. G., and Hutchison, C. J. (1997) GST-lamin fusion proteins act as dominant negative mutants in Xenopus egg extract and reveal the function of the lamina in DNA replication. *J Cell Sci* **110 (Pt 20)**, 2507-2518
42. Adam, S. A., Sengupta, K., and Goldman, R. D. (2008) Regulation of nuclear lamin polymerization by importin alpha. *J Biol Chem* **283**, 8462-8468
43. Hara, Y., and Merten, C. A. (2015) Dynein-Based Accumulation of Membranes Regulates Nuclear Expansion in Xenopus laevis Egg Extracts. *Dev Cell*
44. Benavente, R., Krohne, G., and Franke, W. W. (1985) Cell type-specific expression of nuclear lamin proteins during development of Xenopus laevis. *Cell* **41**, 177-190
45. Yanai, I., Peshkin, L., Jorgensen, P., and Kirschner, M. W. (2011) Mapping gene expression in two Xenopus species: evolutionary constraints and developmental flexibility. *Dev Cell* **20**, 483-496
46. Sun, L., Bertke, M. M., Champion, M. M., Zhu, G., Huber, P. W., and Dovichi, N. J. (2014) Quantitative proteomics of Xenopus laevis embryos: expression kinetics of nearly 4000 proteins during early development. *Sci Rep* **4**, 4365
47. Wuhr, M., Freeman, R. M., Jr., Presler, M., Horb, M. E., Peshkin, L., Gygi, S. P., and Kirschner, M. W. (2014) Deep proteomics of the Xenopus laevis egg using an mRNA-derived reference database. *Curr Biol* **24**, 1467-1475
48. Guo, Y., Kim, Y., Shimi, T., Goldman, R. D., and Zheng, Y. (2014) Concentration-dependent lamin assembly and its roles in the localization of other nuclear proteins. *Mol Biol Cell* **25**, 1287-1297
49. Shimi, T., and Goldman, R. D. (2014) Nuclear lamins and oxidative stress in cell proliferation and longevity. *Adv Exp Med Biol* **773**, 415-430
50. Anderson, D. J., and Hetzer, M. W. (2008) Reshaping of the endoplasmic reticulum limits the rate for nuclear envelope formation. *J Cell Biol* **182**, 911-924
51. Anderson, D. J., and Hetzer, M. W. (2007) Nuclear envelope formation by chromatin-mediated reorganization of the endoplasmic reticulum. Nat Cell Biol 9, 1160-1166
52. Dahl, K. N., Kahn, S. M., Wilson, K. L., and Discher, D. E. (2004) The nuclear envelope lamina network has elasticity and a compressibility limit suggestive of a molecular shock absorber. J Cell Sci 117, 4779-4786
53. Izumi, M., Vaughan, O. A., Hutchison, C. J., and Gilbert, D. M. (2000) Head and/or CaaX domain deletions of lamin proteins disrupt preformed lamin A and C but not lamin B structure in mammalian cells. Mol Biol Cell 11, 4323-4337
54. Kau, T. R., Way, J. C., and Silver, P. A. (2004) Nuclear transport and cancer: from mechanism to intervention. Nat Rev Cancer 4, 106-117
55. Hatch, E., and Hetzer, M. (2014) Breaching the nuclear envelope in development and disease. J Cell Biol 205, 133-141
56. Mall, M., Walter, T., Gorjanacz, M., Davidson, I. F., Nga Ly-Hartig, T. B., Ellenberg, J., and Mattaj, I. W. (2012) Mitotic lamin disassembly is triggered by lipid-mediated signaling. J Cell Biol 198, 981-990
57. Kochin, V., Shimi, T., Torvaldson, E., Adam, S. A., Goldman, A., Pack, C. G., Melo-Cardenas, J., Imanishi, S. Y., Goldman, R. D., and Eriksson, J. E. (2014) Interphase phosphorylation of lamin A. J Cell Sci
58. Abdalla, F., Boder, J., Markus, R., Hashmi, H., Buhmeida, A., and Collan, Y. (2009) Correlation of nuclear morphometry of breast cancer in histological sections with clinicopathological features and prognosis. Anticancer Res 29, 1771-1776
59. Mossbacher, U., Knollmayer, S., Binder, M., Steiner, A., Wolff, K., and Pehamberger, H. (1996) Increased nuclear volume in metastasizing "thick" melanomas. J Invest Dermatol 106, 437-440
60. Wang, N., Stenkvist, B. G., and Tribukait, B. (1992) Morphometry of nuclei of the normal and malignant prostate in relation to DNA ploidy. Anal Quant Cytol Histol 14, 210-216
61. Chow, K. H., Factor, R. E., and Ullman, K. S. (2012) The nuclear envelope environment and its cancer connections. Nat Rev Cancer 12, 196-209
62. Foster, C. R., Przyborski, S. A., Wilson, R. G., and Hutchison, C. J. (2010) Lamins as cancer biomarkers. Biochem Soc Trans 38, 297-300
63. Sun, S., Xu, M. Z., Poon, R. T., Day, P. J., and Luk, J. M. (2010) Circulating Lamin B1 (LMNB1) biomarker detects early stages of liver cancer in patients. J Proteome Res 9, 70-78
64. Lim, S. O., Park, S. J., Kim, W., Park, S. G., Kim, H. J., Kim, Y. I., Sohn, T. S., Noh, J. H., and Jung, G. (2002) Proteome analysis of hepatocellular carcinoma. Biochem Biophys Res Commun 291, 1031-1037
65. Alfonso, P., Canamero, M., Fernandez-Carbonie, F., Nunez, A., and Casal, J. I. (2008) Proteome analysis of membrane fractions in colorectal carcinomas by using 2D-DIGE saturation labeling. J Proteome Res 7, 4247-4255
66. Oguchi, M., Sagara, J., Matsumoto, K., Saido, T., and Taniguchi, S. (2002) Expression of lamins depends on epidermal differentiation and transformation. Br J Dermatol 147, 853-858
67. Coradeghini, R., Barboro, P., Rubagotti, A., Boccado, F., Parodi, S., Carmignani, G., D'Arrigo, C., Patrone, E., and Balbi, C. (2006) Differential expression of nuclear lamins in normal and cancerous prostate tissues. Oncol Rep 15, 609-613
68. Moss, S. F., Krivosheyev, V., de Souza, A., Chin, K., Gaetz, H. P., Chaudhary, N., Worman, H. J., and Holt, P. R. (1999) Decreased and aberrant nuclear lamin expression in gastrointestinal tract neoplasms. Gut 45, 723-729
69. Broers, J. L., Raymond, Y., Rot, M. K., Kuijpers, H., Wagenaar, S. S., and Ramaekers, F. C. (1993) Nuclear A-type lamins are differentially expressed in human lung cancer subtypes. Am J Pathol 143, 211-220
70. Kaufmann, S. H., Mabry, M., Jasti, R., and Shaper, J. H. (1991) Differential expression of nuclear envelope lamins A and C in human lung cancer cell lines. Cancer Res 51, 581-586
71. Skvortsov, S., Schafer, G., Stasyk, T., Fuchsberger, C., Bonn, G. K., Bartsch, G., Klocker, H., and Huber, L. A. (2011) Proteomics profiling of microdissected low- and high-grade prostate tumors identifies Lamin A as a discriminatory biomarker. *J Proteome Res* 10, 259-268

72. Wu, Z., Wu, L., Weng, D., Xu, D., Geng, J., and Zhao, F. (2009) Reduced expression of lamin A/C correlates with poor histological differentiation and prognosis in primary gastric carcinoma. *J Exp Clin Cancer Res* 28, 8

73. Kuramitsu, Y., Hayashi, E., Okada, F., Tanaka, T., Zhang, X., Ueyama, Y., and Nakamura, K. (2010) Proteomic analysis for nuclear proteins related to tumour malignant progression: a comparative proteomic study between malignant progressive cells and regressive cells. *Anticancer Res* 30, 2093-2099

74. Roth, U., Razawi, H., Hommer, J., Engelmann, K., Schwientek, T., Muller, S., Baldus, S. E., Patso, G., Corfield, A. P., Paraskeva, C., and Hanisch, F. G. (2010) Differential expression proteomics of human colorectal cancer based on a syngeneic cellular model for the progression of adenoma to carcinoma. *Proteomics* 10, 194-202

75. Venables, R. S., McLean, S., Luny, D., Morley, S., Quinlan, R. A., Lane, E. B., and Hutchison, C. J. (2001) Expression of individual lamins in basal cell carcinomas of the skin. *Br J Cancer* 84, 512-519

76. Agrelo, R., Setien, F., Espada, J., Artiga, M. J., Rodriguez, M., Perez-Rosado, A., Sanchez-Aguilera, A., Fraga, M. F., Piris, M. A., and Esteller, M. (2005) Inactivation of the lamin A/C gene by CpG island promoter hypermethylation in hematologic malignancies, and its association with poor survival in nodal diffuse large B-cell lymphoma. *J Clin Oncol* 23, 3940-3947

77. Wang, Y., Wu, R., Cho, K. R., Thomas, D. G., Gossner, G., Liu, J. R., Giordano, T. J., Shedden, K. A., Misek, D. E., and Lubman, D. M. (2009) Differential protein mapping of ovarian serous adenocarcinomas: identification of potential markers for distinct tumor stage. *J Proteome Res* 8, 1452-1463

78. Bengtsson, S., Krogh, M., Szigiarto, C. A., Uhlen, M., Schedvins, K., Silfversward, C., Linder, S., Auer, G., Alaiya, A., and James, P. (2007) Large-scale proteomics analysis of human ovarian cancer for biomarkers. *J Proteome Res* 6, 1440-1450

79. Hudson, M. E., Pozdnyakova, I., Haines, K., Mor, G., and Snyder, M. (2007) Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays. *Proc Natl Acad Sci U S A* 104, 17494-17499

FOOTNOTES
The abbreviations used are: NE, nuclear envelope; lamin B3, LB3; lamin B2, LB2; lamin B1, LB1; lamin A, LA; Ig, immunoglobulin; NLS, nuclear localization sequence; ER, endoplasmic reticulum; GFP, green fluorescent protein; MBT, midblastula transition; nuclear pore complex, NPC

FIGURE LEGENDS
FIGURE 1. Lamin B3 concentration affects nuclear size in *X. laevis* egg extract. A) Nuclei were pre-assembled in *X. laevis* egg extract for 30-35 minutes. Recombinant GFP-LB3 was added to the indicated final concentrations, and the same total volume was added to each reaction. For the control reaction, an equal volume of lamin storage buffer was added. Nuclear growth was allowed to proceed for an additional 75 minutes at 20°C. Nuclei were fixed, spun onto coverslips, processed for immunofluorescence, and stained with mAb414 to visualize the NPC (red) and Hoechst to visualize the DNA (blue). Representative images are shown. White arrows denote intranuclear lamin puncta that form upon addition of high concentrations of recombinant lamin proteins. B) For the representative images at the top of the panel, immunofluorescence against GFP was performed, demonstrating that low concentrations of GFP-LB3 properly localize to the NE. The bar graph shows nuclear size data from one representative experiment. For each bar, the cross-sectional areas of 150-870 nuclei were measured from...
NPC-stained nuclei and averaged. The error bars are SD. * p<0.05, ** p<0.01, *** p<0.001, NS Not significant. C) Cumulative data from 20 different X. laevis egg extracts are shown. For each data point, the average cross-sectional nuclear area was measured as in B) and normalized to the buffer addition control (bold horizontal line set at 1.0). The red symbols represent data for LB3 protein without a GFP tag. The error bars are SEM. All normalized nuclear area data points greater than 1.05 and less than 0.94 are statistically different from the buffer control (1.0) by at least p<0.05. Due to variability between egg extracts, the data were normalized to the buffer addition control, and SEM error bars are shown rather than larger SD error bars that obscure visualization and interpretation of the data. D) Similar experiments were performed except that mutant LB3 proteins were added at low (2 nM) or high (34 nM) concentrations. The blue data are for LB3 without a GFP tag. The red data are for GFP-LB3 in which the CAAX box was mutated so it cannot be farnesylated (LB3 amino acids 578 and 579 mutated from cysteines to serines). The green data are for GFP-LB3 with an R385P point mutation within LB3 that renders the protein defective for assembly into the nuclear lamina. For each bar, the cross-sectional areas of 152-944 nuclei were measured from NPC-stained nuclei, averaged, and normalized to the buffer addition control (set at 1.0). The error bars are SEM. * p<0.05, ** p<0.01, *** p<0.001, NS Not significant.

FIGURE 2. Lamin B1, B2, and A concentrations affect nuclear size in X. laevis egg extract. Similar experiments were performed as in Figure 1, using recombinant GFP-LB1, GFP-LB2, and GFP-LA rather than GFP-LB3 (see the Figure Legend for Figure 1). A) Images of representative nuclei. B) Cumulative data from 22 different X. laevis egg extracts are shown. For each data point, the cross-sectional areas of 65-890 nuclei were measured from NPC-stained nuclei, averaged, and normalized to the buffer addition control (bold horizontal line set at 1.0). The open symbols represent data for lamin proteins without a GFP tag. The error bars are SEM. All normalized nuclear area data points greater than 1.06 and less than 0.95 are statistically different from the buffer control (1.0) by at least p<0.05.

FIGURE 3. Lamin combinations affect nuclear size similarly to single lamins in X. laevis egg extract. A) Western blots were performed to estimate the endogenous amounts of different lamins present in X. laevis embryos during development. Known amounts of recombinant lamins were loaded to the left side of each gel (GFP-LB1: 0.45, 0.9, 1.8 pmol; GFP-LB2: 0.5, 1.25, 2.5 pmol; GFP-LA: 0.1, 0.15, 0.2 pmol). 10 µl each of different stage X. laevis embryo extracts were loaded to the right side of each gel. Blots were probed with antibodies previously shown to be specific to each lamin type (data not shown), as well as Ran as a loading control. Absolute protein amounts were determined by comparing band intensities, quantified by infrared fluorescence, to the known amounts of recombinant lamins on the same blot. Embryo extracts prepared from three different frogs were quantified, and one representative western blot is shown for each lamin. Values below each lane represent the mean ± SD (n=3). LB3 amounts were similarly measured previously as 0.75 pmol in the egg, 2.6 pmol in stage 8, 0.3 pmol in stage 11, and negligible in later stages (6). The bar graph shows average total lamin concentrations present at each stage of development with SD error bars (n=3). B) Similar experiments were performed as in Figure 1, except that different lamin combinations were added to nuclei pre-assembled in egg extract (see the Figure Legend for Figure 1). Lamin combinations that approximated stage 8 were: 175 nM LB3 and 50 nM LB1 (blue diamonds). Lamin combinations that approximated stage 11 were: 50 nM LB1 and 50 nM LB2 (red squares). Lamin combinations that approximated stage 19 were: 20 nM LB1, 20 nM LB2, and 5 nM LA (green triangles). Cumulative data from 5 different X. laevis egg extracts are shown. For each data point, the cross-sectional areas of 85-840 nuclei were measured from NPC-stained nuclei, averaged, and normalized to the buffer addition control (bold horizontal line set at 1.0). The error bars are SEM. All normalized nuclear area data points are statistically different from the buffer control (1.0) by at least p<0.01.

FIGURE 4. Lamin concentrations affect nuclear size in X. laevis embryo extracts. A) Embryo extracts containing endogenous embryonic nuclei were prepared from X. laevis embryos at stage 7 to
early stage 8. Recombinant lamins were added to the indicated final concentrations, and the same total volume was added to each reaction. For the control reaction, an equal volume of lamin storage buffer was added. Nuclei were incubated for 60 minutes at 20°C. Nuclei were then fixed, spun onto coverslips, processed for immunofluorescence, and stained with mAb414 to visualize the NPC (red) and Hoechst to visualize the DNA (blue). Representative images are shown. White arrows denote intranuclear lamin puncta that form upon addition of high concentrations of recombinant lamin proteins. B) Nuclear size data from one representative experiment are shown. For each bar, the cross-sectional areas of 370-1242 nuclei were measured from NPC-stained nuclei and averaged. The error bars are SD. * p<0.05, ** p<0.01, *** p<0.001, NS Not significant. From left to right, the number of nuclei quantified/p-values are: 674/not applicable, 459/10^{-23}, 734/10^{-12}, 513/0.03, 1242/10^{-18}, 704/10^{-9}, 616/0.12, 456/10^{-31}, 763/10^{-23}, 882/10^{-10}, 569/10^{-45}, 370/10^{-20}, 605/0.009. C) Cumulative data from 2 different embryo extracts are shown. For each data point, the average cross-sectional nuclear area was measured as in B) and normalized to the buffer addition control (bold horizontal line set at 1.0). The error bars are SEM. All normalized nuclear area data points greater than 1.05 are statistically different from the buffer control (1.0) by at least p<0.05.

FIGURE 5. Lamin concentrations affect nuclear size in vivo in X. laevis embryos. A) Single-cell X. laevis embryos were microinjected with the indicated recombinant lamin proteins or lamin storage buffer as a control. The concentrations on the x-axis represent the final concentration of injected lamins in the embryo assuming an embryo volume of 1 µl. For the lamin combinations, the data point at 36 nM = 15 nM LB1 + 15 nM LB2 + 6 nM LA, and the data point at 55 nM = 30 nM LB1 + 25 nM LB2. Embryos were allowed to develop to early stage 8 and embryo extracts were prepared. Extracts containing endogenous embryonic nuclei were fixed, and nuclei were spun onto coverslips and visualized as described in Figure 1. For each data point, 50-100 embryos were microinjected and the cross-sectional areas of 460-2360 nuclei were measured from NPC-stained nuclei, averaged, and normalized to the buffer injected control (bold horizontal line set at 1.0). The error bars are SEM. * p<0.05, *** p<0.001, NS Not significant. B) Single-cell X. laevis embryos were microinjected with mRNA encoding the indicating lamins or water as a control. Embryos were allowed to develop to stage 11-12 and embryo extracts were prepared. Extracts containing endogenous embryonic nuclei were fixed, and nuclei were spun onto coverslips and visualized as described in Figure 1. Representative images are shown. For the control, nuclei stained for the NPC with mAb414 are shown, while in the other images fluorescently labeled lamins are visualized. C) Nuclear size data from the experimental approach described in B) are plotted as a function of the concentration of ectopically expressed lamins. For each data point, 50-100 embryos were microinjected and the cross-sectional areas of 70-2900 NPC-stained nuclei were measured, averaged, and normalized to the water injected control (bold horizontal line set at 1.0). On the x-axis, the concentrations of ectopically expressed lamin proteins were estimated from infrared fluorescence western blots by comparing the amounts of GFP- or mCherry-tagged lamins in extracts from microinjected embryos to known amounts of recombinant lamin proteins loaded on the same gel (data not shown). The error bars are SEM. All normalized nuclear area data points greater than 1.05 and less than 0.98 are statistically different from the control (1.0) by at least p<0.05.

FIGURE 6. Lamin levels affect nuclear size in mammalian tissue culture cells. A) MRC-5 cells were transiently transfected with plasmids expressing GFP-NLS, GFP-LA, GFP-LB1, or mCherry-LB2. Two days after transfection, cells were transferred to coverslips. After another day, cells were fixed and stained with Hoechst to visualize the DNA (blue). Representative images are shown. B) Nuclear cross-sectional areas were quantified from the red or green channel, depending on the fluorescent label, for transfected MRC-5 cells. For each data point, the cross-sectional areas of 133-608 nuclei were measured and averaged. Error bars are SD. From left to right, the number of nuclei quantified/p-values are: 608/not applicable, 133/10^{-31}, 149/10^{-54}, 215/10^{-62}, 403/not applicable, 163/0.007, 204/10^{-9}, 224/10^{-50}. C) HeLa cells stably expressing GFP-histone H2B were transiently transfected with plasmids expressing GFP-NLS, GFP-LA, GFP-LB1, or mCherry-LB2. Because of the presence of GFP-H2B, all of these plasmids except for mCherry-LB2 were co-transfected with an mCherry plasmid, in order to clearly identify
transfected cells. One day after transfection, cells were transferred to coverslips. After another day, cells were fixed and stained with Hoechst to visualize the DNA (blue). Representative images are shown. For the GFP-LA and GFP-LB1 images, the lamin signal was much stronger than the histone signal, still a weak GFP-H2B signal is visible for all nuclei. Short white arrowheads denote nuclei with intranuclear lamin puncta and/or abnormal nuclear morphology. D) Nuclear cross-sectional areas were quantified from the red or green channel, depending on the fluorescent label, for transfected HeLa cells. For each data point, the cross-sectional areas of 178-2948 nuclei were measured and averaged. Error bars are SD. From left to right, the number of nuclei quantified/p-values are: 2948/not applicable, 195/0.03, 285/10^{-36}, 291/10^{-38}, 178/not applicable, 192/0.13, 356/10^{-6}, 178/10^{-4}, 1006/not applicable, 219/10^{-30}, 362/10^{-38}, 728/10^{-65}. To quantitatively evaluate the level of lamin overexpression, we performed western blots on lysates of transfected HeLa cells (data not shown). Blots were probed with antibodies against LB1, LB2, or LA, as described under Experimental Procedures. To quantify the relative amount of ectopic lamin expression, intensity of the GFP- or mCherry-tagged lamin band was divided by the intensity of the endogenous lamin band. The amount of ectopic lamin expression for Experiments 1, 2, and 3, respectively, was 56%, 27%, 63% for LB1; 35%, 23%, 78% for LB2; 20%, 10%, 20% for LA. For Experiment 4, HeLa cells were transiently transfected with plasmids expressing LA, LB1, or LB2 and co-transfected with an mCherry plasmid to allow for identification of transfected cells. Nuclear cross-sectional areas were quantified from Hoechst-stained cells. E) HeLa cells were transiently transfected with siRNA against the indicated lamin or a control siRNA with no target in the human genome. Cells were co-transfected with an mCherry plasmid to allow for identification of transfected cells. One day after transfection, cells were transferred to coverslips. After another day, cells were fixed and stained with Hoechst. Representative images of Hoechst-stained nuclei are shown. White arrows denote nuclei in cells that received the indicated siRNA, as determined by co-transfection with the mCherry plasmid and corresponding mCherry fluorescence (not shown). (F) For each data point, the cross-sectional areas of 105-1418 nuclei in transfected cells were measured and averaged. From left to right, the number of nuclei quantified/p-values are: 549/not applicable, 229/10^{-13}, 445/10^{-25}, 445/10^{-79}, 1418/not applicable, 163/10^{-13}, 251/10^{-10}, 269/10^{-5}, 1085/not applicable, 105/10^{-54}, 160/10^{-50}, 111/10^{-51}. Error bars are SD. * p<0.05, ** p<0.01, *** p<0.001, NS Not significant.

FIGURE 7. Cumulative Xenopus nuclear size data plotted as a function of total lamin concentration. Blue diamonds represent all in vitro data from experiments with egg extracts (Fig. 1C, 2B, 3B), red squares represent all in vitro and in vivo data from experiments with early stage embryos (Fig 4C, 5A), and green triangles represent all in vivo data from stage 11-12 embryos (Fig. 5C). The x-axis is total lamin concentration estimated by summing the ectopic lamin concentration (either added recombinant protein or protein expressed from microinjected mRNA) plus the endogenous lamin concentration for that stage of development.
Figure 2

A) Lamins added to egg extract containing pre-assembled nuclei

| Lamin concentration | Control | 0.5 nM GFP-LB1 | 100 nM GFP-LB1 | 1 nM GFP-LB2 | 100 nM GFP-LB2 | 1 nM GFP-LA | 45 nM GFP-LA |
|---------------------|---------|----------------|----------------|--------------|----------------|--------------|--------------|
| NPC (red)           |         |                |                |              |                |              |              |
| Hoechst             |         |                |                |              |                |              |              |
| GFP                 |         |                |                |              |                |              |              |
| MERGE               |         |                |                |              |                |              |              |

10 µm

B) Graph showing nuclear area normalized to buffer control vs. lamin concentration added to egg extract (nM)

Lamin added:
- GFP-LB1: blue diamonds
- GFP-LB2: red squares
- GFP-LA: green triangles
- LB1: blue diamonds
- LB2: red squares
- LA: green triangles
Figure 3

A

| Embryonic Stage | pmol ± SD |
|-----------------|-----------|
| 8               | 0.8 ± 0.4 |
| 11              | 0.4 ± 0.1 |
| 15              | 0.5 ± 0.2 |
| 19              | 0.4 ± 0.1 |
| 28              | 0.6 ± 0.3 |

B

Lamin combination that mimics:
- Stage 19
- Stage 11
- Stage 8

Total lamin concentration added to egg extract (nM)
Figure 4

A) Lamin addition to pre-MBT embryo extract containing endogenous nuclei

| Lamin concentration added (nM) | Control | 1 nM GFP-LB3 | 2 nM GFP-LB3 | 6 nM GFP-LB3 | 12 nM GFP-LB3 | 63 nM GFP-LB3 | 0.2 nM GFP-LB2 | 100 nM GFP-LB1 | 100 nM GFP-LB1 | 1 nM GFP-LA | 100 nM GFP-LA |
|--------------------------------|---------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|-------------|--------------|
| NPC (red)                      |         |               |               |               |               |               |               |               |               |             |              |
| Hoechst                        |         |               |               |               |               |               |               |               |               |             |              |
| GFP                             |         |               |               |               |               |               |               |               |               |             |              |
| MERGE                           |         |               |               |               |               |               |               |               |               |             |              |

10 μm

B) Representative experiment

Cross-sectional nuclear area (μm²)

C) Cumulative data

Lamin added:
- GFP-LB3
- GFP-LB2
- GFP-LB1
- GFP-LA

Nuclear area normalized to buffer control

Lamin concentration added (nM)

Cumulative data

Lamin concentration added (nM)
**Figure 5**

**A**

Early stage 8

![Graph showing nuclear area normalized to buffer injected control with final lamin protein concentration injected into embryos (nM)]

- **Final lamin protein concentration injected into embryos (nM):**
  - GFP-LB3: 1, 38
  - GFP-LB2: 1, 25
  - GFP-LB1: 1, 44
  - GFP-LA: 1, 50
  - Combinations: 36, 55

**B**

mRNA injected at stage 1, nuclei isolated and visualized at stage 11-12

- control (mAb414)
- GFP-LA
- GFP-LB1
- mCherry-LB2
- GFP-LB3

- **Images:**
  - Control (mAb414)
  - GFP-LA
  - GFP-LB1
  - mCherry-LB2
  - GFP-LB3

- **Scale:** 20 μm

**C**

Stage 11-12

![Graph showing nuclear area normalized to control injected embryos with concentration of lamin ectopically expressed from microinjected mRNA (nM)]

- **mRNA injected:**
  - GFP-LB3
  - mCherry-LB2
  - GFP-LB1
  - GFP-LA

- **Concentration of lamin ectopically expressed from microinjected mRNA (nM):**
  - 50 to 500
Figure 6

**A**

MRC-5 cells

| Transfected plasmid: | MRC-5 cells |
|----------------------|-------------|
| GFP-NLS              | **GFP-LB1** | **mCherry-LB2** |

| Lamin (green or red) |  |
|----------------------|--|
| GFP-LA               | |
| GFP-LB1              | |
| mCherry-LB2          | |

| Hoechst (blue)       |  |
|----------------------|--|
| GFP-LA               | |
| GFP-LB1              | |
| mCherry-LB2          | |

| MERGE                |  |
|----------------------|--|
| GFP-LA               | |
| GFP-LB1              | |
| mCherry-LB2          | |

20 µm

**B**

Transfected plasmid:

MRC-5 cells

Cross-sectional nuclear area (µm²)

| Experiment | GFP-NLS | GFP-LB1 | mCherry-LB2 |
|------------|---------|---------|-------------|
| 1          | **100** | 200     | 300         |
| 2          | **100** | 200     | 300         |

*** p < 0.001
**  p < 0.01
*  p < 0.05
NS

**C**

HeLa cells

| Transfected plasmid: | HeLa cells |
|----------------------|------------|
| GFP-NLS              | **GFP-LB1** | **mCherry-LB2** |

| Lamin (green or red) |  |
|----------------------|--|
| GFP-LA               | |
| GFP-LB1              | |
| mCherry-LB2          | |

| Hoechst (blue)       |  |
|----------------------|--|
| GFP-LA               | |
| GFP-LB1              | |
| mCherry-LB2          | |

| MERGE                |  |
|----------------------|--|
| GFP-LA               | |
| GFP-LB1              | |
| mCherry-LB2          | |

20 µm

**D**

Transfected plasmid:

HeLa cells

Cross-sectional nuclear area (µm²)

| Experiment | GFP-NLS | GFP-LB1 | mCherry-LB2 |
|------------|---------|---------|-------------|
| 1          | **100** | 200     | 300         |
| 2          | **100** | 200     | 300         |
| 3          | **100** | 200     | 300         |
| 4          | **100** | 200     | 300         |

*** p < 0.001
**  p < 0.01
*  p < 0.05
NS

**E**

HeLa cells

| Transfected plasmid: | HeLa cells |
|----------------------|------------|
| Control siRNA        | **LA siRNA** |
|                     | **LB1 siRNA** |
|                     | **LB2 siRNA** |

| Hoechst               |  |
|-----------------------|--|
| Control siRNA         | |
| LA siRNA              | |
| LB1 siRNA             | |
| LB2 siRNA             | |

20 µm

**F**

HeLa cells

Cross-sectional nuclear area (µm²)

| Experiment | Control | LA | LB1 | LB2 |
|------------|---------|----|-----|-----|
| 1          | **100** | 200| 300 | 300 |
| 2          | **100** | 200| 300 | 300 |

*** p < 0.001
**  p < 0.01
*  p < 0.05
Figure 7

Cumulative nuclear size data from all *Xenopus* in vitro and in vivo experiments

- **Egg extract**
- **Pre-MBT embryo extract**
- **Post-MBT embryos**

**Normalized nuclear area** vs. **Total lamin concentration, endogenous + ectopic (nM)**
Concentration-Dependent Effects of Nuclear Lamins on Nuclear Size in *Xenopus* and Mammalian Cells
Predrag Jevtic, Lisa J. Edens, Xiaoyang Li, Thang Nguyen, Pan Chen and Daniel L. Levy

*J. Biol. Chem.* published online October 1, 2015

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