Soft Matter

Nuclear stiffening and chromatin softening with progerin expression leads to an attenuated nuclear response to force

Journal:	*Soft Matter*
Manuscript ID:	SM-ART-03-2015-000521.R2
Article Type:	Paper
Date Submitted by the Author:	30-Jun-2015
Complete List of Authors:	Booth, Elizabeth; Carnegie Mellon University, Chemical Engineering Spagnol, Stephen; Carnegie Mellon University, Chemical Engineering Alcoser, Turi; Carnegie Mellon University, Biomedical Engineering Dahl, Kris; Carnegie Mellon University,

Soft Matter

RSCPublishing

ARTICLE

Nuclear stiffening and chromatin softening with progerin expression leads to an attenuated nuclear response to force

Cite this: DOI: 10.1039/x0xx00000x

E. A. Booth*[a], S. T. Spagnol*[a], T. A. Alcoser[b] and K. N. Dahl [a,b]

DOI: 10.1039/x0xx00000x

www.rsc.org/

Progerin is a mutant form of the nucleoskeletal protein lamin A, and its expression results in the rare premature aging disorder Hutchinson-Gilford progeria syndrome (HGPS). Patients with HGPS demonstrate several characteristic signs of aging including cardiovascular and skeletal dysfunction. Cells from HGPS patients show several nuclear abnormalities including aberrant morphology, nuclear stiffening and loss of epigenetic modifications including heterochromatin territories. However, it is unclear why these changes disproportionately impact mechanically-responsive tissues. Using micropipette aspiration, we show that nuclei in progerin-expressing cells are stiffer than control cells. Conversely, our particle tracking reveals the nuclear interior becomes more compliant in cells from HGPS patients or with progerin expression, as consistent with decreased chromatin condensation as shown previously. Additionally, we find the nuclear interior is less responsive to external mechanical force from shear or compression likely resulting from damped force propagation due to nucleoskeletal stiffening. Collectively our findings suggest that force is similarly transduced into the nuclear interior in normal cells. In HGPS cells a combination of a stiffened nucleoskeleton and softened nuclear interior leads to mechanical irregularities and dysfunction of mechanoresponsive tissues in HGPS patients.

A Introduction

Hutchinson-Gilford progeria syndrome (HGPS) is a premature aging disorder caused by a mutation in lamin A, a structural protein of the cell nucleus. The mutant lamin A, also called Δ50 lamin A or progerin, accumulates at the nuclear envelope[1] leading to downstream nuclear defects. These defects in nuclei of cells from patients with HGPS include abnormal morphology,[2] thickening of the nuclear lamina [1] and stiffening of the nucleus.[3] Additionally, these cells exhibit increased DNA damage[4] and altered chromatin modification patterns.[5] Specifically, heterochromatin appears to be decondensed in cells from patients with HGPS.[6] However, heterochromatin is a load-bearing element in nuclei[7] and, in some cases, dictates the stiffness of the entire nucleus.[8-10] Thus, the precise nature of the nuclear stiffening from progerin within the context of the integrated nucleoskeleton and chromatin mechanical network is not well understood.

Systemically, effects of HGPS are most pronounced in structural and force-responsive tissues. These include endothelial cells exposed to shear stress[11] and skeletal tissues under compression[12] as well as many others. Shear stress is of particular interest for study in patients with progeria due to increased incidence of atherosclerosis[13] stemming from endothelial cell dysfunction and an improper genetic response to shear stress.[14-16] However, it is unclear how a mutant protein expressed in most cells of the body has its most pronounced pathological effects primarily in force-responsive tissue types. Also, how nuclear stiffening could cause this progressive, segmented syndrome is currently unknown.

The role of physiological forces, including shear stress and compression, in stimulating changes in gene expression has been well established.[17-19] In addition to mechanotransduction in the cell membrane, focal adhesions and cytoskeleton, the nucleus acts as a mechanoresponsive element within the cell. Recent work suggests that direct mechanical force propagation from the cell into the nuclear interior via the LINC (LInker of Nucleus to Cytoskeleton) complex alters nuclear structure and dynamics and may facilitate the underlying chemical signaling mechanisms.[20-23] Also, evidence of force-induced changes to chromatin condensation state[24] and enhanced force sensitivity in euchromatin relative to heterochromatin[20] provides plausible

mechanisms for the role of mechanical force in chromatin reorganization.

Here, we study the stiffness of nuclei using the traditional mechanical measurement technique micropipette aspiration (MPA) and find that nuclei of cells exogenously expressing progerin are stiffer than control counterparts, similar to cells from patients with HGPS. However, in measuring the compliance of the chromatin within the nucleus, we find that the chromatin is softer inside cells expressing progerin. Thus, the composite structure of the nucleus shows a stiffened nucleoskeleton, but softer chromatin interior with the expression of progerin. Further, we show that forces, from both the cytoskeletal molecular motors or from external stresses imposed on the cell, have a reduced effect on chromatin movements in cells expressing progerin. This suggests that progerin expression in HGPS alters the mechanical connections of the cell, and may explain altered cell function and gene expression in the mechanical tissues of patients.

B Materials and Methods

Cell Culture, Transfection and Characterization

Human cervical cancer cells (HeLa, ATCC) and human osteosarcoma cells (Saos2, ATCC) were cultured using Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific) with 10% fetal bovine serum (Life Technologies, Grand Island, NY) and 1% penicillin-streptomycin (Life Technologies). Human umbilical vein endothelial cells (HUVECs, ATCC) were cultured using endothelial base media with growth supplements (Lonza). Fibroblasts were generously donated from the Progeria Research Foundation (Peabody, MA) and were cultured in DMEM with high glucose, 15% fetal bovine serum, 1% L-glutamine and 1% penicillin-streptomycin (Life Technologies). Two cells lines from the Progeria research foundation were used: an HGPS cell line (HGADFN167, referred to as HGPS) with the mutation CT608 in exon 11, and an adult control (HGADFN168, referred to as control) from a 40-year-old parent, negative for the point mutation. Cells were imaged to determine localization of exogenous lamins as well as confirm HGPS-related morphologies. See supplemental methods, supplemental results, Figures S1-S3 and Table S1.

Cells were transfected with recombinant DNA of fluorescent tagged upstream binding factor one (UBF1-GFP[25]) or fibrillarin (Fib-GFP, a kind gift from D. Dischер, University of Pennsylvania). HeLa cells were transfected with Polyfect (Qiagen) per manufacturer's recommendations, media was changed, and cells were incubated an additional 24 hours before imaging. Saos2 cells and HUVECs were transfected with Lipofectin (Life Technologies) and media was changed after 5-8hrs of transfection. All cells experienced 48 hrs of incubation post-transfection prior to imaging.

Micropipette Aspiration

MPA experiments were performed on wild type cells and cells expressing GFP-UBF1 and DsRed-progerin. Micropipettes were pulled from 1 mm glass capillaries using a PMP102 Micropipette puller (Microdata). The ends of the micropipette tips were between 3 and 10 μm in diameter. Cells were trypsized and resuspended in a PBS solution with 3 μM of cytochalasin D (Sigma-Aldrich), 0.125 μM of Nocodazole (Sigma-Aldrich) and 0.17 μg/mL of Hoescht 33342 to label the nuclei (Life Technologies). Nocodazole inhibits polymerization of microtubules and cytochalasin D inhibits actin polymerization. A micromanipulator (Narishige) was used to approach the cells of interest. A pressure was then applied and the cells were aspirated into the pipette. Pressure was applied through a syringe and passes from air to a water reservoir that runs parallel to a pressure transducer (Validyne) and the pipette that is in contact with the cell of interest. Any change in pressure was given time to equilibrate at which time an image was captured, then the pressure was increased and the process repeated. The effective whole nuclear stiffness (E_{eff}) was computed from the micropipette radius (R_p), the change in pressure (ΔP), and the length of the cell up the pipette (L_P).

$$\Delta P \propto E_{eff} \frac{L_P}{R_P} \qquad \text{(Equation 1)}$$

Shear and Compressive Force

For shear experiments, transfected cells were passed into a micro-slide VI flow chamber (ibidi) 24 hours prior to a shear exposure. The shear stress was applied from a peristaltic pump (Instech) through two media reservoirs to buffer the flow and then through the ibidi micro-slide. Media was pre-equilibrated to 37°C and 5% CO_2 for a minimum of 30 minutes, and the entire flow apparatus was housed inside a Pecon live-cell imaging chamber on the microscope. Nuclei were labeled with 1 μg/mL Hoechst 33342 (Life Technologies) and incubated for a minimum of 30 minutes. Images of cells were captured at multiple sites per experiment using an automated stage every 3 minutes. Cell viability was confirmed by continued imaging for over an hour after the completion of data collection. Shear stress of 20 dyn/cm^2 was used for all shear experiments.

During compression experiments, transfected cells were seeded into 35 mm MatTek dishes. The compressive force was applied by a 100 g weight set on top of a glass coverslip above the cells, leading to a total force of 1.0 N. Nuclei were labeled with 1 μg/mL Hoescht 33342 and with 0.3 μg/ml propidium iodide (Life Technologies, Grand Island, NY) to indicate cell death and incubated for a minimum of 30 minutes prior to starting the experiments. Images were acquired every 2 minutes, and cell viability was confirmed by propidium iodide staining and continued imaging for over an hour after the completion of data collection.

Cells were imaged on an inverted microscope (DMI6000, Leica) using a 63x (1.4 NA) oil immersion objective. During imaging the entire microscope environment was regulated by a Pecon live-cell imaging chamber heated to 37°C. Cells were viable beyond 2 hours, the duration of the experiment. The time

steps used in these experiments were determined in order to account for phototoxity and photobleaching over the course of the experiments.

Particle tracking analysis

Data analysis was done as consistent with previous studies.[26-28] Particle tracking image analysis was done with custom Laptrack71 suite of programs designed in Matlab.[29] Images were initially aligned to remove noise caused by cellular drift, translocation, and rotation. The images were then statistically segmented to select for bright spots and the spot information was restructured into particle tracks (Figure S4). Mean squared displacement (MSD) was calculated for the first hour of data taken (Equation 2) where t is time, τ is the time lag, and x and y are the position coordinates at the given times.

$$MSD = \langle (x_{t+\tau} - x_t)^2 + (y_{t+\tau} - y_t)^2 \rangle \quad \text{(Equation 2)}$$

$$MSD = J_{eff}\tau^{\alpha} \quad \text{(Equation 3)}$$

The chromatin dynamics measured by MSD exhibit power-law time dependence (Equation 3). Further characterization of the nature of these dynamics comes from the fitting parameters. Previous studies of cellular manipulation by biological agents have shown that the fitting parameters are separable and indicative of cellular events. Specifically, J_{eff} is a measure of the effective compliance of the intranuclear chromatin network, where chromatin decondensation enhances the effective compliance and *vice versa* increasing the amplitudes of the motion within the nucleus.[28] By contrast, α indicates the level of system forces driving these dynamics including motor proteins that enhance motion beyond thermal energy and external force application.[28] We have summarized the control data from previous studies showing the impact of known chromatin compliance and system forces on particle tracking results in Figure 1.[28]

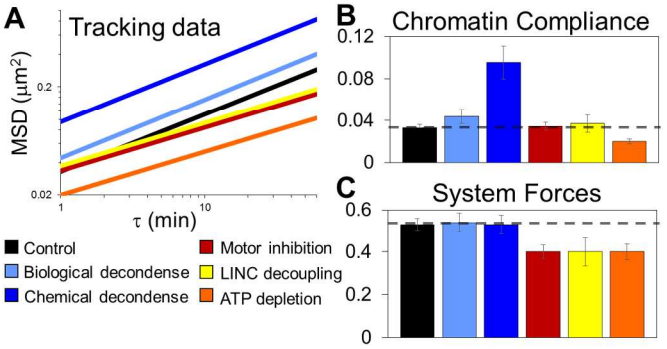

Figure 1: Summary of previous control studies of nuclear particle tracking and discrete impacts on chromatin compliance and system forces.[28] (A) Fits of experimental data to MSD versus τ on a log-log graph yield straight lines. (B) The intercept, J_{eff} from Equation 3, is modulated by decondensation of chromatin by trichostatin A (biological) and daunomycin (chemical). (C) Conversely, system forces, α from Equation 3, are altered by myosin inhibiting Blebbistatin, dominant negative KASH domain protein and ATP depletion.

Particle tracking statistics

Between 5 and 15 different nuclei from 2-3 independent experiments were analysed for each data set with when calculating MSD. For all fitted parameters of "chromatin compliance" (J_{eff}) and "system forces" (α), statistical significance is determined by students T-test with $*p<0.05$ and error bars are 95% confidence intervals.

C Results

Exogenous progerin expression stiffens nuclei

Previously, nuclei isolated from HGPS patient fibroblasts were shown to be stiffer than control nuclei.[3] To test if the exogenous expression of progerin has the same nuclear stiffening effect, we investigated the effect of DsRed-progerin expression in HeLa cells using the same technique: micropipette aspiration (MPA). We characterized localization of exogenously expressed progerin and nuclear morphological differences associated with progerin expression, and we showed exogenous expression was similar HGPS pathology (Figure S1, S3, Table S1). Expression of progerin causes some dysmorphic formations in nuclei including invaginations, creases, and blebbing of the nuclear lamina (Figure S2). When considering large numbers of samples, nuclei either showed no statistical difference from control or small differences in lamin localization and shape (Table S1), as seen previously.[30] The effect of exogenous UBF1-GFP (used in subsequent experiments) was monitored by co-transfection. Most human nuclei deform viscoelastically.[31] However, our results demonstrate that HeLa nuclei deform elastically under MPA-imposed forces on short time scales (on the order of seconds) with increasing pressure causing increased deformation into the micropipette, as observed previously in HeLa cells.[32]

Control HeLa cells displayed an effective whole nuclear stiffness of ~4 kPa (Figure 2). In contrast, progerin expression resulted in an effective whole nuclear stiffness of more than double that of control cells of ~9 kPa**.** This suggests that the overexpression of progerin is sufficient to significantly stiffen the nucleus, as seen in patient cells.[3]

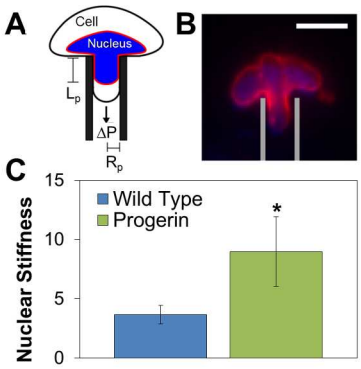

Figure 2: Exogenous progerin expression stiffens nuclei. A) Schematic of micropipette aspiration (MPA) of the nucleus within a cell. B) MPA was performed on whole HeLa cells with depolymerized cytoskeleton and nuclei labeled with Hoechst 33342. An example aspirated nuclei expressing DsRed-progerin. Scale bar is 10 μm. C) With increasing pressure, nuclear deformation was determined from nuclear stretch into the micropipette. DsRed-progerin-expressing cells are approximately 2.4 times stiffer than the other control cell populations. n>6 for all measurements. Error bars are mean squared error (SEM) *p<0.05.

Progerin expression reduces chromatin condensation and softens the nuclear interior

The stiffness of the nucleus is governed both by the stiffness of the lamina nucleoskeleton as well as the chromatin interior. Since progerin accumulates at the nuclear envelope,[1] we next sought to examine how progerin expression affects the chromatin dynamics and mechanics of the nuclear interior. To do this we expressed chromatin-bound, GFP-tagged probes (UBF1-GFP or Fib-GFP since chromatin dynamics are probe independent[27, 28]) and tracked the movements in live cells (for trajectories see Figure S4). With image processing to remove cellular movements, we were able to calculate the intranuclear dynamics of chromatin (mean squared displacement or MSD). MSD versus lag time values were then fit to the model in Equation 3 to determine quantifiable parameters for chromatin compliance and system forces driving chromatin movement. MSD can be decoupled into "Chromatin Compliance" as a measure of chromatin condensation and "System Forces" as a measure of myosin II motor activity on the cytoskeleton directed through the LINC complex into the nuclear interior (see Methods, Figure 1 and previous work[28] for details).

Imaging of particle tracks in cells from HGPS patients and control cells show different behaviour (Figure S6). By fitting data to the model and examining the time-independent factor (J_{eff}, Equation 3), we find a statistically significant increase in the chromatin compliance of HGPS cells over control (Figure 3). This is similar to other studies that have shown that chromatin in HGPS nuclei is less condensed than control cells.[6]

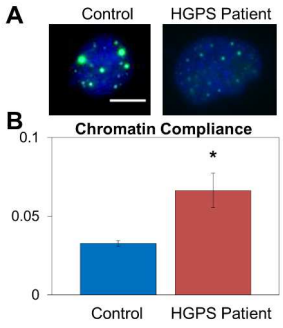

Figure 3 HGPS patient cells show enhanced chromatin compliance. (A) Punctate regions of GFP-tagged proteins used for particle tracking with Hoechst 33342-stained DNA in control patient (left) and HGPS patient cells (right). Scale bar is 10 μm. (B) HGPS patient cells have increased chromatin compliance relative to patient control cells indicating reduced chromatin condensation in HGPS patients. For raw MSD data see Figure S6.

In cells from patients with HGPS, chromatin compliance was significantly increased compared with matched controls (Figure 3B). Similarly, exogenous expression of DsRed-progerin resulted in increased chromatin compliance for HeLa cells (Figure 4D), HUVECs (Figure 4E) and Saos2 cells (Figure 4F). Thus, our results all show that progerin expression consistently causes increased compliance (effective softening) of the nuclear interior. Comparison with control experiments (Figure 1) suggests that progerin expressing cells have reduced chromatin condensation. Further, these results indicate that the stiffening of the nucleus in progerin-expressing cells is associated with a stiffened nucleoskeleton and not the nuclear interior.

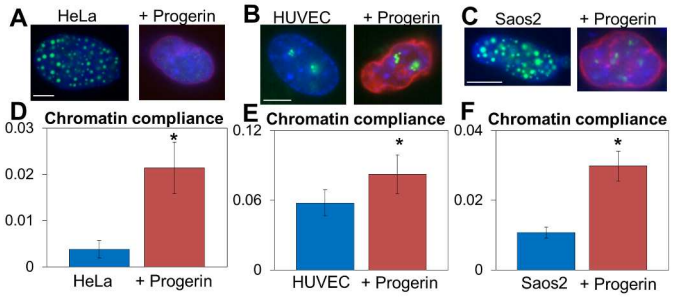

Figure 4: Exogenous expression of DsRed-Progerin enhances chromatin compliance in cells. Punctate regions of GFP-tagged proteins used for particle tracking Hoechst 33342 labelled nuclei in (A) HeLa cells, (B) HUVECs and (C) Saos2 cells with and without DsRed-Progerin. Exogenous progerin expression increased chromatin compliance in (D) HeLa cells, (E) HUVECs and (F) Saos2 cells relative to controls indicating reduced chromatin condensation. Scale bars are 10 μm. For raw MSD data see Figures S7-S9.

Progerin expression reduces propagation of cytoskeletal forces to the nuclear interior

From the fits of MSD, it is also possible to determine system forces that providing the driving force for chromatin dynamics (α from Equation 3). In cells at rest these enhanced system forces above thermal motion are derived from molecular motors, primarily the cytoskeletal molecular motors myosin II, propagated to the nuclear interior that augment movements of the chromatin as well as mechanically communicating force from the cell to the nuclear interior.[20, 28] All cells expressing progerin, including HGPS patient cells, had a reduction in system forces, most of which were significant (Figure 5). Previously we have shown that force transmission from the cytoskeleton to the nuclear interior can be inhibited by interrupting the LINC complex that connects the cytoskeleton to the nucleoskeleton (Figure 1). Here, we suggest that an alteration of cytoskeletal-nuclear mechanical transduction can also be altered with a stiffening of the nucleoskeleton. Progerin

expression and nucleoskeletal alterations may damp force propagation into the nuclear interior from the cytoskeleton.

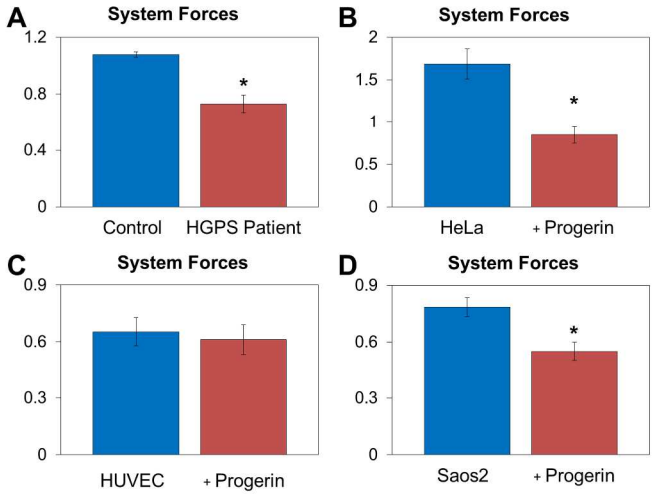

Figure 5: Progerin expression reduces cytoskeletal force propagation to the nuclear interior. System forces as measured from the particle tracking experiments indicate the level of cytoskeletal forces and external forces propagated to the nuclear interior. Progerin expression in patient cells (A) as well as exogenous expression in HeLa cells (B), HUVECs (C) and Saos2 cells (D) results in reduced system forces from reduced force propagation to the nuclear interior. For raw MSD data see Figures S6-S9.

Progerin expression also reduces the intranuclear response to extracellular applied force

HGPS pathology is most pronounced in mechanically active tissues where the progerin-induced dysfunction results in aberrant mechanosensing. As such, we aimed to investigate progerin-expressing cells under physiologically relevant force. Since progerin-expressing cells are less able to transduce force from the cytoskeleton to the nuclear interior, we were interested in how this affects the intranuclear response to external force. We tracked motion of chromatin in live cells under stress using fluid shear stress on endothelial cells (HUVEC) and compression on bone cells (Saos2). The stress type was matched to the cell type since endothelial cells are responsive to shear stress in blood vessels and bone cells are responsive to compressive stress in bone.

As expected, application of stress to control cells resulted in an increase in "System Forces", additive above the actin-myosin system forces, driving enhanced chromatin movement compared to the stress-free cells (Figure 6C-D). In this case, the increased force propagation to the nuclear interior is derived from the myosin motors and applied stress. Application of stress also reduced chromatin compliance (Figure 6A-B). There are numerous reasons that this could occur; we suggest the possibility that this change occurs from nuclear compression and reduced nuclear volume.[24]

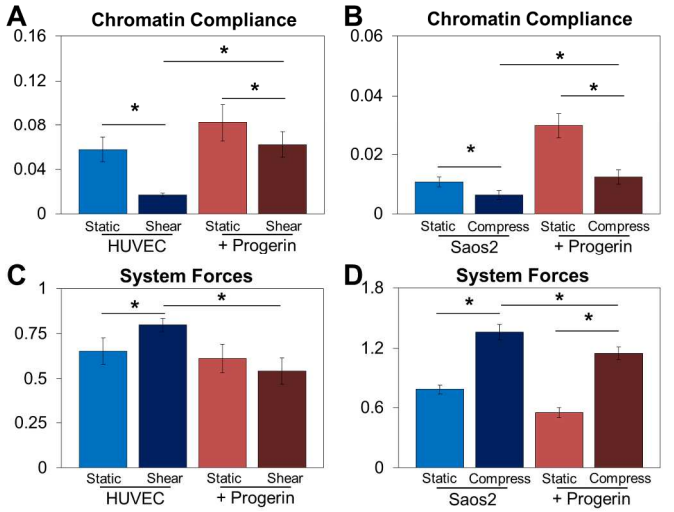

Figure 6: Progerin expression reduces intranuclear mechanical sensitivity to external force application. External force application from 20 dyn/cm^2 shear stress to HUVECs or 1 N compression to Saos2 cells results in reduced chromatin compliance (A and B, respectively) while increasing system forces propagated to the nuclear interior (C and D, respectively) in control cells. By contrast, while progerin-expressing cells experienced reduced chromatin compliance upon external force application relative to static progerin-expressing cells, it fails to recapitulate the chromatin condensation state of control cells under shear (A) or compression (B). Further, progerin-expressing HUVECs under shear experience no increase in system forces relative to static progerin-expressing cells (C) while progerin-expressing Saos2 cells under compression do not experience the same degree of system forces propagated to the nuclear interior as control cells under compression (D). For MSD data see Figures S8 and S9.

In progerin-expressing cells exposed to the same stresses, we observed an attenuated response. We again observed a decrease in chromatin compliance with applied stress (Figure 6A-B), but the progerin-expressing HUVEC cells were less responsive than control cells. More dramatically, the progerin-expressing HUVECs showed no statistical change in system forces with shear stress application (Figure 6C). This suggests these extracellular applied forces are not significantly transduced to the nuclear interior with exogenous progerin expression. Progerin-expressing Saos2 cells under compression also showed a statistically attenuated response to stress application (statistical difference when comparing Saos2 cells under force with and without progerin, Figure 6D). However, the impacts of progerin expression are less detrimental in the case of compression, likely because the large force is applied normal to the cells. Generally, these results suggest that progerin expression results in an attenuated intranuclear mechanical

response to external force likely due at least in part to nucleoskeletal stiffening.

D Discussion

Previously, nuclear stiffening has been observed in HGPS patient fibroblasts.[3, 33] Here, we show that exogenous progerin expression also causes stiffening of the whole nucleus, as measured by MPA. While progerin expression stiffens the nucleus when measured as a whole structure, particle tracking experiments of chromatin bound probes indicate the nuclear interior softens as a result of progerin expression in both patient cells and exogenously expressing cells. Our group and others have shown that particle tracking can show changes in intranuclear rheology associated with cell treatment,[34-37] complementing high strain alternatives such as MPA. Thus, nucleoskeletal stiffening seems to be the predominant contributor to the increased nuclear stiffness observed in progerin-expressing cells. We suggest that this is consistent with the preferential localization of progerin to the nuclear envelope resulting from farnesyl tail association with the membrane,[3] whereas wild type lamin A can exchange between the nucleoplasm and the lamina.[3, 38]

Progerin expression has previously been shown to reduce chromatin condensation in heterochromatin territories labelled by H3K9me3 and HP1α and in silenced inactivated X chromosomes.[6] Progerin-induced dysregulation of genome organization includes altered chromatin tethering at the nuclear periphery,[39] the mislocalization of telomeres and the clustering of centromeres.[13] We similarly find an overall reduction in chromatin condensation associated with progerin expression, and our work highlights the impact on chromatin dynamics that may further impact this dysregulation. Analysis of our chromatin dynamics data suggests that the enhanced chromatin compliance we observe with progerin expression is a manifestation of the loss of higher order chromatin organization. This likely results in a loss of genome function, where aberrant transcriptional activity[40] and increased incidence of DNA damage and compromised repair are associated with progerin expression.[41-43] It is of particular concern with DNA damage, where increased chromatin dynamics may be implicated in translocation frequency.[44, 45] Given HGPS patient cells exhibit elevated levels of reactive oxygen species (ROS) causing increased DNA damage,[43] further genomic instability imparted by increased chromatin dynamics may act to compound the problem.

HGPS patients experience dysfunction associated with mechanoresponsive tissues including bone degradation and cardiovascular complications, with heart attacks and strokes being the most common fatalities. Previous studies have shown that progerin-expressing cells are more mechanosensitive and prone to apoptosis in response to mechanical stress.[33] Our results provide some mechanistic details suggesting why mechanosensitive tissues show altered gene expression patterns. Specifically, we find the nuclear interior of progerin expressing cells to have a reduced response to forces, either from the cell's own molecular motors or from extracellular applied force. In the most dramatic example, we observe no change in force propagation to the chromatin in progerin-expressing HUVECs under shear whereas control cells showed shear stress related changes.[27] Thus, it seems progerin expression reduces the natural mechanical force propagation to the nuclear interior that is necessary for the normal mechanical response.

In normal individuals, a number of genes expressed in endothelial cells and smooth muscle cells under shear stress are thought to confer an "atheroprotective" phenotype inhibiting atherosclerosis.[46] The attenuated intranuclear response to force (as evidenced in the muted chromatin dynamics) in progerin-expressing cells resulting from progerin accumulation may play a role by inhibiting the mechanical signalling required for chromatin reorganization and expression. To this end, recent work has highlighted the role of direct mechanical force transduction in mediating biochemical signalling cascades through force-induced chromatin decondensation and increased fluctuations.[20, 28] Further, nuclear transport of some transcription factors has been shown to depend on the presence of intact cytoskeletal-nuclear mechanical structures, with transport coinciding with cytoskeletal reorganization and chromatin remodelling.[20] Thus, it seems likely that the attenuated genetic response of progerin-expressing cells to mechanical stimulus may follow directly from the reduced force transduction into the nuclear interior,[47] which we show definitively contributes to reduced chromatin dynamics relative to control cells.

The increased stiffness of the nucleoskeleton is the most obvious rationale for the reduced force transduction in progerin-expressing cells. We suggest that the presence of a disproportionately stiffer nucleoskeleton would damp forces transmitted from outside the nucleus. However, progerin expression also alters nucleoskeletal connections to the LINC complex,[48] which would further limit force transduction. We have also shown recently that HGPS patient cells have reduced traction force generation[49] and other studies indicate decreased cytoskeletal stiffness,[50, 51] both of which likely contribute to the loss of cell polarity observed and the reduced ability to align in the flow direction under shear. These hallmarks of the disease – reduced cytoskeletal force generation, loss of proper LINC connections and a stiffened nucleoskeleton – likely result in the reduced system forces driving chromatin dynamics in progerin-expressing cells even under static conditions where less cytoskeletal force is transmitted to the nuclear interior. More broadly, our findings suggest a complete collapse of mechanical integrity in progerin-expressing cells. These results highlight that the nucleus and cytoskeleton operate as a wholly-integrated mechanical network that operates bi-directionally to facilitate nuclear structure and genome function on one side and to balance force generation for adhesion and motility on the

other. As such, dysfunction in a single component results in loss of proper chromatin organization and dynamics as well as nucleoskeletal and cytoskeletal mechanical integrity. Thus, HGPS provides a model system that demonstrates a necessary role for mechanical mechanisms as regulators of genome function and stability, the absence of which provides insight into the pathologies of cardiovascular disease and DNA damage.

Acknowledgements

We gratefully acknowledge Ge Yang (Carnegie Mellon Biomedical Engineering, Lane Center for Computational Biology and Biological Sciences) and Minhua Qui (Carnegie Mellon Biomedical Engineering) for development and assistance with the LAPtrack71 suite of particle tracking programs. This work is supported by the NSF (CBET-0954421 and CMMI-1300476 to KND).

Notes and references

*These authors contributed equally

[a] Department of Chemical Engineering, Carnegie Mellon University.

[b] Department of Biomedical Engineering, Carnegie Mellon University.

† Electronic Supplementary Information (ESI) available: Supplemental methods, results and images include characterization of protein localization and nuclear morphology as well as image processing and raw data of particle tracking MSD versus τ for experimental samples. See DOI: 10.1039/b000000x/

1. R. D. Goldman, D. K. Shumaker, M. R. Erdos, M. Eriksson, A. E. Goldman, L. B. Gordon, Y. Gruenbaum, S. Khuon, M. Mendez, R. Varga and F. S. Collins, *Proceedings of the National Academy of Sciences of the United States of America*, 2004, **101**, 8963-8968.
2. P. Scaffidi and T. Misteli, *Science*, 2006, **312**, 1059-1063.
3. K. N. Dahl, P. Scaffidi, M. F. Islam, A. G. Yodh, K. L. Wilson and T. Misteli, *Proceedings of the National Academy of Sciences of the United States of America*, 2006, **103**, 10271-10276.
4. B. Liu, J. Wang, K. M. Chan, W. M. Tjia, W. Deng, X. Guan, J. D. Huang, K. M. Li, P. Y. Chau, D. J. Chen, D. Pei, A. M. Pendas, J. Cadinanos, C. Lopez-Otin, H. F. Tse, C. Hutchison, J. Chen, Y. Cao, K. S. Cheah, K. Tryggvason and Z. Zhou, *Nat Med*, 2005, **11**, 780-785.
5. Y. Gruenbaum, A. Margalit, R. D. Goldman, D. K. Shumaker and K. L. Wilson, *Nat Rev Mol Cell Biol*, 2005, **6**, 21-31.
6. D. K. Shumaker, T. Dechat, A. Kohlmaier, S. A. Adam, M. R. Bozovsky, M. R. Erdos, M. Eriksson, A. E. Goldman, S. Khuon, F. S. Collins, T. Jenuwein and R. D. Goldman, *Proceedings of the National Academy of Sciences of the United States of America*, 2006, **103**, 8703-8708.
7. A. Mazumder and G. V. Shivashankar, *Journal of the Royal Society, Interface / the Royal Society*, 2010, **7 Suppl 3**, S321-330.
8. K. N. Dahl, A. J. Engler, J. D. Pajerowski and D. E. Discher, *Biophys J*, 2005, **89**, 2855-2864.
9. A. Mazumder and G. V. Shivashankar, *Biophysical journal*, 2007, **93**, 2209-2216.
10. A. Harikumar and E. Meshorer, *Methods in molecular biology*, 2013, **1042**, 173-180.
11. D. McClintock, L. B. Gordon and K. Djabali, *Proc Natl Acad Sci U S A*, 2006, **103**, 2154-2159.
12. C. Sanchez, L. Pesesse, O. Gabay, J. P. Delcour, P. Msika, C. Baudouin and Y. E. Henrotin, *Arthritis Rheum*, 2011, **64**, 1193-1203.
13. P. Taimen, K. Pfleghaar, T. Shimi, D. Moller, K. Ben-Harush, M. R. Erdos, S. A. Adam, H. Herrmann, O. Medalia, F. S. Collins, A. E. Goldman and R. D. Goldman, *Proc Natl Acad Sci U S A*, 2009.
14. R. M. Nerem, *J Biomech Eng*, 1992, **114**, 274-282.
15. O. Traub and B. C. Berk, *Arterioscler Thromb Vasc Biol*, 1998, **18**, 677-685.
16. K. M. Eyster, S. E. Appt, C. J. Mark-Kappeler, A. Chalpe, T. C. Register and T. B. Clarkson, *Menopause*, 2011, **18**, 1087-1095.
17. A. R. Brooks, P. I. Lelkes and G. M. Rubanyi, *Physiological genomics*, 2002, **9**, 27-41.
18. A. R. Brooks, P. I. Lelkes and G. M. Rubanyi, *Endothelium-J Endoth*, 2004, **11**, 45-57.
19. A. C. Shieh and K. A. Athanasiou, *Osteoarthr Cartilage*, 2007, **15**, 328-334.
20. K. V. Iyer, S. Pulford, A. Mogilner and G. V. Shivashankar, *Biophysical journal*, 2012, **103**, 1416-1428.
21. D. N. Simon and K. L. Wilson, *Nat Rev Mol Cell Bio*, 2011, **12**, 695-708.
22. N. Wang, J. D. Tytell and D. E. Ingber, *Nat Rev Mol Cell Bio*, 2009, **10**, 75-82.
23. A. J. Maniotis, C. S. Chen and D. E. Ingber, *Proceedings of the National Academy of Sciences of the United States of America*, 1997, **94**, 849-854.
24. M. Versaevel, T. Grevesse and S. Gabriele, *Nature communications*, 2012, **3**, 671.
25. J. D. Pajerowski, K. N. Dahl, F. L. Zhong, P. J. Sammak and D. E. Discher, *Proceedings of the National Academy of Sciences of the United States of America*, 2007, **104**, 15619-15624.
26. G. Yang, L. A. Cameron, P. S. Maddox, E. D. Salmon and G. Danuser, *Journal of Cell Biology*, 2008, **182**, 631-639.
27. E. A. Booth-Gauthier, T. A. Alcoser, G. Yang and K. N. Dahl, *Biophys J*, 2012, **103**, 2423-2431.
28. S. T. Spagnol and K. N. Dahl, *Integr Biol-Uk*, 2014, **6**, 523-531.
29. G. Yang, A. Matov and G. Danuser, *IEEE Computer Society Conference on Computer Vision and Pattern Recognition (CVPR'05)*, 2005.
30. S. Choi, W. Wang, A. J. Ribeiro, A. Kalinowski, S. Q. Gregg, P. L. Opresko, L. J. Niedernhofer, G. K. Rohde and K. N. Dahl, *Nucleus*, 2011, **2**, 570-579.
31. K. N. Dahl, S. M. Kahn, K. L. Wilson and D. E. Discher, *J Cell Sci*, 2004, **117**, 4779-4786.
32. A. C. Rowat, L. J. Foster, M. M. Nielsen, M. Weiss and J. H. Ipsen, *J R Soc Interface*, 2005, **2**, 63-69.
33. V. L. R. M. Verstraeten, J. Y. Ji, K. S. Cummings, R. T. Lee and J. Lammerding, *Aging Cell*, 2008, **7**, 383-393.

34. I. B. Berger, E. Kepten and Y. Garini, *Methods in molecular biology*, 2013, **1042**, 139-151.
35. B. Sinha, D. Bhattacharya, D. K. Sinha, S. Talwar, S. Maharana, S. Gupta and G. V. Shivashankar, *Methods in cell biology*, 2010, **98**, 57-78.
36. V. Levi and E. Gratton, *Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology*, 2008, **16**, 439-449.
37. A. Zidovska, D. A. Weitz and T. J. Mitchison, *Proceedings of the National Academy of Sciences of the United States of America*, 2013, **110**, 15555-15560.
38. J. L. Broers, B. M. Machiels, G. J. van Eys, H. J. Kuijpers, E. M. Manders, R. van Driel and F. C. Ramaekers, *Journal of cell science*, 1999, **112 (Pt 20)**, 3463-3475.
39. N. Kubben, M. Adriaens, W. Meuleman, J. W. Voncken, B. van Steensel and T. Misteli, *Chromosoma*, 2012, **121**, 447-464.
40. M. Columbaro, C. Capanni, E. Mattioli, G. Novelli, V. K. Parnaik, S. Squarzoni, N. M. Maraldi and G. Lattanzi, *Cellular and Molecular Life Sciences*, 2005, **62**, 2669-2678.
41. P. Scaffidi and T. Misteli, *Nat Cell Biol*, 2008, **10**, 452-459.
42. E. K. Benson, S. W. Lee and S. A. Aaronson, *Journal of cell science*, 2010, **123**, 2605-2612.
43. S. A. Richards, J. Muter, P. Ritchie, G. Lattanzi and C. J. Hutchison, *Human molecular genetics*, 2011, **20**, 3997-4004.
44. V. Roukos, T. C. Voss, C. K. Schmidt, S. Lee, D. Wangsa and T. Misteli, *Science*, 2013, **341**, 660-664.
45. V. Dion and S. M. Gasser, *Cell*, 2013, **152**, 1355-1364.
46. J. Zhou, Y. S. Li and S. Chien, *Arteriosclerosis, thrombosis, and vascular biology*, 2014, **34**, 2191-2198.
47. M. L. Lombardi and J. Lammerding, *Biochem Soc Trans*, 2011, **39**, 1729-1734.
48. F. Haque, D. Mazzeo, J. T. Patel, D. T. Smallwood, J. A. Ellis, C. M. Shanahan and S. Shackleton, *Journal of Biological Chemistry*, 2010, **285**, 3487-3498.
49. E. A. Booth-Gauthier, V. Du, M. Ghibaudo, A. D. Rape, K. N. Dahl and B. Ladoux, *Integr Biol-Uk*, 2013, **5**, 569-577.
50. C. M. Hale, A. L. Shrestha, S. B. Khatau, P. J. Stewart-Hutchinson, L. Hernandez, C. L. Stewart, D. Hodzic and D. Wirtz, *Biophys J*, 2008, **95**, 5462-5475.
51. C. Y. Ho, D. E. Jaalouk, M. K. Vartiainen and J. Lammerding, *Nature*, 2013, **497**, 507-+.

