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Physical Plasticity of the Nucleus and its Manipulation

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Abstract

The genome is virtually identical in all cells within an organism, with epigenetic changes contributing largely to the plasticity in gene expression during both development and aging. These changes include covalent modifications of chromatin components and altered chromatin organization as well as changes in other nuclear components, such as nuclear envelope lamins. Given that DNA in each chromosome is centimeters long and dozens of chromosomes are compacted into a microns-diameter nucleus through non-trivial interactions with the bounding envelope, the polymer physics of such a structure under stress can be complex but perhaps systematic. We summarize micromanipulation methods for measuring the physical plasticity of the nucleus, with recent studies documenting the extreme flexibility of human embryonic stem cells and the rigidification in model aging of progerin-type nuclei. Lamin-A/C is a common molecular factor, and methods are presented for its knock-down and measurement.

I. Introduction

The nucleus is generally the largest single organelle of a eukaryotic cell and is literally the cell's defining feature. Within the nuclei of a given organism, the DNA is essentially identical, but the many differentiated cell types possess epigenetic differences that fix the fates of progenitors and stem cells. DNA methylation, histone isoforms, and histone modifications collectively regulate gene expression but so do nuclear envelope proteins that also change in development. For example, lamin-A/C is largely absent from human embryonic stem cells (Constantinescu *et al.*, 2006), but spliced isoforms accumulate at the nuclear periphery in cells from aged, normal humans (Scaffidi and Misteli, 2006). We hypothesized that such epigenetic plasticity in normal development would be mechanically measurable and meaningful. A rigid nucleus, for example, would likely imply less accessible genes and a more terminally differentiated cell fate.

A methodology was therefore sought that could lend physical insight into nuclear development as well as various physiological and technological processes. For example, blood capillaries at 2–3 μm in diameter are similar in size or smaller than nuclei, which means that nuclei in cells ranging from white blood cells to circulating meta-static cancer cells must be able to deform and flow in order to access peripheral tissues. Micropipette aspiration is a standard method to understand the flow and deformation of cells into tubes (Fig. 1). In terms of technology motivations, somatic cell nuclear transfer (SCNT) involves micropipette manipulation of a nucleus from an adult somatic cell for transfer into a denucleated, unfertilized egg (Wakayama *et al.*, 1998). SCNT techniques are notoriously inefficient (Wilmut and Paterson, 2003) and might benefit from a better fundamental understanding of stress effects on nuclei and their substructures.

II. Micropipette Aspiration

A. Basic Experimental Method

This method has several important features: (1) the length scale of deformation is similar in length scale to nuclear subdomains or “nuclear territories” (Lieberman-Aiden *et al.*, 2009), (2) the change in nuclear shape under stress can be measured over a wide range of time, and (3) the deformation modes of the different nuclear subcomponents labeled fluorescently (with DNA dyes or GFP-constructs) can be visualized by fluorescence microscopy. With a nucleus that is isolated from a cell (see below), aspiration reveals nuclear responses without the affects of physical links between the nucleus and different components of the cytoskeleton. Solution conditions are readily changed, including both ionic strength and osmotic strength that control nuclear volume and molecular interactions (Dahl *et al.*, 2005). However, measurements made on nuclei within intact cells can in principle provide more relevant *in situ* insight into the physical behavior of the nucleus provided the cytoskeleton and other cell components do not interfere. This has been achieved when necessary by depolymerizing filamentous actin in cells with latrunculin immediately prior to aspiration (Pajerowski, 2007). Whether the nucleus is isolated or not, it is partially aspirated into a micropipette with a preset pressure and the increase in the projection length of the nucleus is measured as a function of time. The key steps are as follows:

1. Micropipettes are prepared from a 1-mm-diameter glass capillary by pulling with a micropipette puller and following methods that are standard in electrophysiology. Typically, the inner diameter is between 1 and 5 μm , and this should be constant over at least 10 μm of length in order to visualize the aspirated projection of nucleus. This is achieved by optimization of the pulling rate.
2. The micropipettes are fractured and forged to create a flat tip. This is important to ensure symmetric aspiration and flow of the nucleus into the micropipette.
3. The micropipette inner and/or outer surface can be then passivated to minimize cell or nuclear adhesion and friction. This is achieved by immersing into albumin solutions or silanizing solutions.
4. The micropipette is backfilled with physiological buffer using a syringe with a suitably small gauge needle.
5. The micropipette is mounted on a micromanipulator connected to a pressure-controlled system in which the applied pressure (negative relative to atmospheric) is measured by a calibrated pressure transducer or manometer.
6. Using the micromanipulators, the micropipette is positioned horizontally within the focal plane of the microscope and close to the nucleus to be aspirated. After the nucleus is slightly aspirated, the micropipette should be raised above the coverslip which the cell or nucleus is settled upon in order to avoid friction between the nucleus and the supportive surface during the aspiration.
7. Negative pressures from -1 to -10 kPa are required for aspiration of the nucleus. Higher pressures should be avoided because they can rupture the nucleus. In the case of measurements of a nucleus within a cell, repetitive aspiration of the cell can be used to mechanically disrupt the cell wall and thus isolate the nucleus (Guilak, 2000).

B. Mathematics of Physical Responses

1. The DNA in each of the 46 human chromosomes is a massive macromolecule (~ 1 – 10 cm long) that is condensed as chromatin into a microns-diameter nucleus and would be expected—as with most polymers—to exhibit complex flow behavior

(i.e., complex rheology). While some past studies of nuclear deformation have assumed fully recoverable elastic behavior (Deguchi *et al.*, 2005), others have suggested viscoelastic behavior (Guilak, 2000) or shown more complex power law rheology (Dahl *et al.* 2005, Pajerowski, 2007).

For small deformations most solid materials can be described by Hooke's law of linear elasticity in which E is Young's elastic modulus for the material. The one-dimensional relationship between the applied stress σ and caused strain ε is given by

$$\sigma = E\varepsilon$$

The inverse of the modulus E is called compliance:

$$J = \frac{1}{E}$$

A purely viscous fluid under shear obeys the following relationship between the stress and strain rate:

$$\sigma = \eta \frac{d\varepsilon}{dt}$$

where η is the viscosity. Real materials often deviate from pure elastic or viscous behavior and exhibit a more complex and time-dependent stress versus deformation response. If they yield or break after some period of stress and thereafter do not recover their deformation, then they are referred to as "plastic."

Creep is defined as a progressive deformation of a material held under constant stress. If a sudden stress is applied to a material (Fig. 2A), then it can be described with

$$\sigma = \sigma_0 H(t)$$

where $H(t) = 0$ for $t < 0$ and $H(t) = 1$ for $t > 0$. The compliance might then prove time dependent and is called the creep compliance

$$J(t) = \frac{\varepsilon(t)}{\sigma_0}$$

Creep curves can capture material behaviors over many decades of time, but if the load is released the material might begin to recover. If the recovery never reaches zero strain, then the remaining strain is an indication of the plasticity of the material. Linear viscoelastic materials are those for which the creep compliance is independent of the stress.

On the other hand, if the strain is suddenly imposed and held constant, then one can present the strain history as a step function (Fig. 2B)

$$\varepsilon = \varepsilon_0 H(t)$$

A decrease in stress in a material held under constant strain is called relaxation and can be described with relaxation modulus

$$E(t) = \frac{\sigma(t)}{\varepsilon_0}$$

Creep and relaxation function can be experimentally obtained and their mathematical descriptions involve attempts to capture a solid–liquid duality.

Differential constitutive models relate the stress and the strain in linear differential equations with constant coefficients by connecting the basic elements—elastic (springs) and viscous (dashpots) in different ways. A classical Maxwell model consists of a spring and dashpot connected in series with a constitutive equation that reads:

$$\frac{d\varepsilon}{dt} = \frac{1}{E} \frac{d\sigma}{dt} + \frac{\sigma}{\eta}$$

A spring and dashpot connected in parallel (Kelvin–Voigt model) has the form:

$$\sigma = E\varepsilon + \eta \frac{d\varepsilon}{dt}$$

For elements in series, the stresses coincide with the total strain being a sum of the elemental strains. For elements in parallel, the strains coincide and the total stress is a sum of stresses in the individual elements. This approach can be used to design more complicated models by combining different Maxwell and Voigt elements in parallel and/or in series to capture the complex rheological behavior of the biological materials.

2. Complexity in Fractional Differential Models. More complex rheological properties may be expressed in terms of fractional derivatives. An element might obey an equation of the form

$$\sigma(t) = E\tau_r^\beta \frac{d^\beta \varepsilon(t)}{dt^\beta}$$

where E is the stiffness, τ_r is a relaxation time constant, and β is a material-dependent parameter can be used to obtain the relaxation function $E(t)$. In such a case, the relaxation function is a power law in time that may be approximated by

$$E(t) = Bt^{-n}$$

Then using the Laplace transform it can be shown that the creep function is also a power law.

$$J(t) = At^n$$

For $n=0$ the compliance is independent of time, which is the case for purely elastic response, and for $n=1$, the response is purely viscous. For any n 's that are between these extremes, the viscoelastic complexity of the material can be captured. Power-law models describe adequately the creep and relaxation function of many materials with small number of adjustable parameters and over of many decades of time.

3. Nuclear Creep in Micropipette Aspiration. For the particular case of micropipette aspiration of a nucleus that is much larger than the micropipette diameter, the creep of a nucleus into the micropipette can be measured as

$$J(t) = \frac{2\pi\Phi}{3} \frac{1}{P} \frac{\Delta L(t)}{R_p}$$

where Φ is a geometry-dependent numerical prefactor (~ 2.1), P is the constant pressure applied by the micropipette, and the strain is given by ΔL , which is the dynamic aspirated length normalized to the pipette radius R_p . This equation for $J(t)$ provides the instantaneous measurable creep; nuclei could aspirate independent of time or exhibit a more complex power-law response.

Micropipette measurements performed on both stem cells and differentiated cells over time scales of about 100 s or more (Fig. 1B and C) tend to exhibit a power-law creep compliance with creep exponents of $n \sim 0.2$ – 0.6 (Pajerowski, 2007). After approximately 10 s, the deformation proves irreversible, and this provides clear evidence of the plasticity of the nucleus, that is, a permanent rearrangement of the nucleus and its chromatin. In general, the nuclear compliance of fully differentiated and aged cells (fibroblast and epithelial cells) is demonstrably lower than that in younger cells, including both human hematopoietic stem cells (HSCs) and pluripotent human embryonic stem cells (hESCs). The latter nuclei, over several days in differentiation media, exhibit a sixfold increase in stiffness (Fig. 1B). Visualization of the chromatin in differentiated cells with GFP-histones show that the chromatin is pinned at places to the nuclear envelope; the chromatin therefore extends and flows into the aspirating micropipette.

Micropipette aspiration of fibroblast nuclei from patients with an accelerated aging laminopathy further show that, particularly with “aging” by passage in culture, nuclei rigidify (Fig. 1C and D) with differences in young versus old mean rheological parameters of about 20–30%. The images of GFP-lamin not only illustrate the slightly reduced extension of the aged nucleus into the micropipette after 200 sec but further show how the nuclear envelope folds and wrinkles outside the aspirating micropipette. Detailed molecular studies of nuclei from aged normal donors versus young donors suggest accumulation of a particular deletion isoform of lamin-A at the nuclear envelope in aged cells (Scaffidi and Misteli, 2006). This seems consistent with the fact that lamin-A/C is not expressed in the ESCs nor in HSCs.

III. Molecular Mechanisms from Reengineered Nuclei

To clarify the relative contributions of different nuclear components to nuclear plasticity and rheology, a systematic perturbation of specific molecules is required. While B-type lamins appear expressed in all cell types, lamin-A/C varies considerably as mentioned above, suggesting that knockdown of lamin-A/C in a differentiated cell type such as an epithelial cell might test more directly the role of this lamin in nuclear mechanics. Results from

micropipette aspiration confirm the hypothesized role for lamin-A/C, as elaborated elsewhere (Pajeroski, 2007). Other nuclear molecules might be approached similarly.

A. Lamin Knockdown with RNA Interference

RNA interference (RNAi) is a phenomenon whereby double-stranded RNA induces posttranscriptional silencing of target mRNA by catalytic cleavage in a relatively sequence-specific manner. RNAi was first discovered in *Caenorhabditis elegans* (Fire *et al.*, 1998), and it was later shown that synthetic 21–23 nucleotide RNA termed short interfering RNA (siRNA) could silence in cultured mammalian cells (Elbashir *et al.*, 2001). When siRNA is delivered to the cytoplasm, it is incorporated in a multiprotein complex called the RNA-induced silencing complex (RISC). Once in the RISC, the sense strand of siRNA is cleaved, and the remaining antisense strand directs the complex to the target mRNA. After binding of the siRNA antisense strand to its target, mRNA is catalytically cleaved by one subcomponent of the RISC. This process is called posttranscriptional gene silencing. Since its discovery in the late 1990s, RNAi has been intensively studied not only for understanding function of genes but also for clinical applications (Castanotto and Rossi, 2009). Here we outline the methods of lamin-A/C downregulation using siRNA and the commercially available transfection agent Lipofectamine 2000, with subsequent analysis of knockdown efficiency. We note here that similar methods can be used for transfections of GFP-lamins and other nuclear proteins (Pajeroski, 2007).

1. siRNA Complex for Transfection—Different types of transfection reagents are commercially available. Lipofectamine 2000 (LF2k) is a cationic lipid-based nanosized particle that complexes with nucleic acids (lipoplex) through electrostatic interactions when mixed in an appropriate buffer. Excess cationic charge of the complex also promotes cell uptake by binding to the net-negative cell membrane. Cargo release into cytoplasm occurs, it is thought, via an osmotic rupture of endolysosomes.

Preparation of LF2k/siRNA complex follows the protocol provided by Invitrogen (San Diego, CA, USA). Dilute LF2k and siRNA (sequence) in the same volume of Opti-MEM (Invitrogen): normally 20 µg/ml LF2k in 50 µl and siRNA 20-fold of desired concentration in 50 µl.

- Incubation for 15 min at room temperature.
- Mix two solutions in one and incubate for 15 min at room temperature.

2. Transfection of Adherent Epithelial Cells—A549 lung epithelial cells are seeded 24 h prior to transfection. High-glucose DMEM (Invitrogen) supplemented with 10% FBS is used for cell culture and transfection. Before adding the siRNA complex, cells are washed with DPBS (Invitrogen) once and provided with fresh medium. Complex solution was added such that complex/medium = 1/10 in volume. Cells are incubated 72 h in 37°C humidified chamber with 5% CO₂.

3. Immunostaining for Lamin-A/C Visualization—This protocol is standard immunostaining as provided by Abcam (<http://www.abcam.com/>).

- The A549 cells are seeded in 12-well plates (Corning) with 10,000 cells per well 24 h prior to transfection.
- After transfection is done, fix cells with 3.7% formaldehyde (Fisher Scientific) in DPBS. Incubate for 15 min at room temperature.
- Wash cells with ice-cold DPBS twice.

- Permeabilize cells with 0.25% Triton X-100 (MP Biomedicals) in DPBS. Incubate for 10 min at room temperature.
- Incubate cells with DPBS for 5 min at room temperature 3 times.
- Incubate cells with 1% bovine serum albumin (BSA, Sigma-Aldrich) in phosphate-buffered saline (PBS) with 0.05% Tween-20 (Fisher Scientific). Incubation time is 30 min in 37°C humidified chamber with 5% CO₂.
- Dilute primary antibody against lamin-A/C (mouse monoclonal IgG, Santa Cruz Biotech) 200 times in DPBS with 1% BSA and 0.25% Tween-20 (concentration of antibody is 1 µg/ml). Add 400 µl of 200× antibody solution to each well and incubate either 1 h in 37°C humidified chamber with 5% CO₂ or overnight at 4°C.
- After incubation with primary antibody, incubate cells with DPBS at room temperature for 5 min. Repeat this wash step 3 times.
- Dilute Alexa Fluor 488-conjugated donkey-derived antimouse polyclonal antibody (Invitrogen) 200 times in DPBS with 1% BSA and 0.25% Tween-20 (concentration of antibody is 10 µg/ml). Incubate cells with secondary antibody (400 µl in each well) for 1 h in 37°C humidified chamber with 5% CO₂. 10 min before finishing incubation, add 4 µl of 50 times-diluted Hoechst 33342 dye (Invitrogen) suspended in DPBS.
- After incubation with secondary antibody and Hoechst 33342, incubate cells with DPBS at room temperature for 5 min. Repeat this step 3 times.
- Cells are imaged by epifluorescence microscopy (Olympus IX71) using a 40 × objective lens.
- Image collection on any sensitive charge-coupled device camera under constant image settings for knockdown and control cells can be used to quantify the relatively lower intensity of lamin-A/C in the knockdown cells. DNA staining with Hoechst can be used to also determine the DNA intensity in any given nucleus as a lamin measurement, and then a scatterplot of intensities can be made: lamin versus DNA. The knockdown nuclei should be a scattering of points below the non-transfected cells. Typical knockdown levels are 50% or more, with considerable cell-to cell variation.

4. Western Blotting for Lamin-A/C Knockdown—Standard protocols are also available (<http://www.abcam.com/>).

- 10⁶ cells were seeded in 60-mm cell culture dish (Falcon) 24 h prior to transfection. Total volume of medium and sample is 2 ml.
- After 72 h, detach the cells by 1.5 ml of trypsin with 0.05% EDTA (Invitrogen). Wash cells with ice-cold DPBS twice (centrifugation is done with 5000 rpm for 5 min at 4°C).
- After removing DPBS, add 250 µl of NP40 lysis buffer (1% NP40 + 50 mM Tris Base + 150 mM NaCl) with 1% protease inhibitor (Sigma-Aldrich). Incubate on ice for 30 min with occasional vortex.
- During the incubation, sonicate sample for 15 s.
- Centrifuge the sample with 12,000 rpm for 20 min at 4°C and collect supernatant.
- For 200 µl lysate, add 66 µl of Laemmli buffer (Invitrogen) and 6 µl of β-mercaptoethanol. Heat the sample at 80–90°C for 5–10 min.

- Prior to the electrophoresis, determine the total protein concentration of the lysate using BCA Protein Assay Kit (Pierce).
- Load sample with the volume based on the aimed amount of total protein per each well. Run time is 10 min with 100 V and 65 min with 160 V. It is best to run several different protein concentrations from both knockdown and control for later analysis.
- Blot protein on PVDF membrane using iBlot system (Invitrogen).
- Block membrane with 10% nonfat dry milk (American Analytical) in TTBS (4.6 mM Tris Base, 15.4 mM Tris HCl, 154 mM NaCl and 0.1% Tween-20) on a rocker at room temperature for 1 h.
- Wash membrane with TTBS once for 15 min and twice for 5 min on rocker.
- Cut the membrane such that it includes approximately 60–80 kDa proteins. Incubate the membrane in a solution of 400-fold diluted primary antibody against lamin-A/C (mouse-derived, Santa Cruz Biotechnology) in TTBS. When using β -actin as a loading control, cut out the membrane between 40 and 50 kDa and incubate it in 1000-fold diluted solution of β -actin primary antibody (mouse-derived, Santa Cruz Biotechnology). Incubation can be done either at room temperature for 1–2 h or at 4°C overnight.
- Wash membrane with TTBS once for 15 min and twice for 5 min on rocker.
- Dilute antimouse HRP-conjugated IgG (GE Healthcare) 2000 times in TTBS with 5% dry milk. Incubate membranes in secondary antibody solution with rocking at room temperature for 1 h.
- Wash membrane with TTBS once for 15 min and twice for 5 min, and then with TBS (4.6 mM Tris Base, 15.4 mM Tris HCl, 154 mM NaCl) on rocker.
- Develop with Chromosensor (GenScript) for 2–3 min at room temperature.
- Scan developed membrane and analyze by densitometry using Image J. A plot of immunostaining intensity versus protein load should fit to a line, and the slope of the knockdown should be lower than that of the control cells.

IV. Isolation of Individual Nuclei

Although manipulation results cited above have appeared largely consistent between in-cell nuclei and isolated nuclei (Dahl *et al.*, 2005), whenever there is concern that the cell is contributing unduly to the apparent nuclear properties, nuclear isolation should be attempted. Spheroidal nuclei from at least some cell types can be isolated by both single-cell mechanical extraction (Guilak, 2000) and bulk methods (Caille *et al.*, 2002; Dahl *et al.*, 2005; Deguchi *et al.*, 2005). Extremely fragile nuclei have not yet been isolated successfully, and since the nuclear envelope breaks down during mitosis, the methods apply only to mechanically stable interphase nuclei. Isolation also takes advantage of the nucleus' relative rigidity and its tenuous connections to the rest of the cell.

One detailed protocol for bulk isolation, described in detail previously (Dean and Kasamatsu, 1994) and shown to yield nuclei suited to micromechanical characterization (Dahl *et al.*, 2005), is briefly described below. It has the advantage that further biochemical analyses such as proteomic profiling can be applied to the nuclei (Andersen *et al.*, 2005; Black *et al.*, 2007), perhaps even under stressed conditions (Johnson *et al.*, 2007). In general, the cell's plasma membrane is disrupted mechanically with hypotonic swelling or chemically with digitonin or other surfactants that perturb the plasma membrane but not

nuclear membranes. The cell is then opened with mechanical homogenization, and cellular contents are separated from the nuclei by ultracentrifugation through a sucrose gradient.

A. Bulk Isolation Protocol

1. Stock solutions

- “10× TKMC”: 500 mM Tris, 250 mM KCl, 25 mM MgCl₂, 30 mM CaCl₂; adjust
- to approximately pH 8.
- “2.3 STKMC”: dilute 10-fold, 2.3 M sucrose, adjust to pH 7.6 at 4°C.
- “5× STKMC”: dilute 2-fold, 1.25 M sucrose, adjust to pH 7.6 at 4°C.
- 10 mM HEPES, pH 7.5.

Prepare on the day of use (cool to 4°C):

- 200 µl 5× STKMC, 2.5 µl protease inhibitor cocktail (PIC).
- 3 ml 2.3 STKMC, 7.5 µl PIC.
- Take 900 µl of 2.3 STKMC and add BSA to make 0.2 mg/ml (“2.3 STKMC–BSA”).
- 10 ml of 10 mM HEPES, pH 7.5 with 1 mM DTT (dithiothreitol).

2. Harvesting nuclei

- Cells should be nearing confluency: 10⁵ cells minimum, 10⁸ maximum. Wash the T75 flask with 5 ml cold PBS. Repeat.
- Wash once with 5 ml cold HEPES/DTT buffer.
- Scrape cells and make volume up to 1 ml with HEPES/DTT buffer.
- Cool on ice for 10 min.
- 25 strokes in Dounce Homogenizer (avoiding air bubbles).
- Take 900 µl of homogenized cells, add 180 µl 5× STKMC.
- Cool on ice for 10 min.
- Add 2 ml 2.3 STKMC; ensure solution is well mixed (sucrose concentration is now 1.6 M).
- Pipette 200 µl 2.3 STKMC–BSA into each of four ultracentrifuge tubes (polycarbonate 8 × 34 mm²).
- Layer 750 µl cell lysate into each tube (the interface between the solutions will be visible).
- Spin for 1 h at 50,000 rpm in the TLS 55 rotor at 4°C. The nuclei should pellet at the bottom, other cell material should collect at the gradient interface.
- Remove the supernatant and resuspend the pellet.

3. At this point nuclei can be resuspended into any media, although adding BSA helps break up clumps and facilitates manipulation later. Nuclei can be counted relatively accurately on a hemacytometer. Subsequent to isolation, it is important to assess the quality of the nuclei by comparison with nuclei in intact cells, and to make sure

that the nuclear envelope remains intact while avoiding excess membranes such as the endoplasmic reticulum or cytoskeletal structures. DNA stains such as Hoechst dyes or, better, GFP-lamins allow fluorescence visualization for assessment of nuclear morphology.

4. Imaging of isolated nuclei

- Resuspend nuclei in 50 μ l 10 mM Tris with 10,000:1 diluted Hoescht stain.
- Incubate at 37°C for 10 min.
- Centrifuge to pellet the nuclei, then pipette away the supernatant.
- Resuspend in 50 μ l 10 mM Tris with 500:1 diluted phalloidin–rhodamine stain.
- Incubate at 37°C for 30 min.
- Centrifuge to pellet, pipette away supernatant, resuspend in 50 μ l 10 mM Tris.
- Examine nuclei by fluorescence microscopy, including immunostaining for lamin-A/C as outlined above.

5. Lastly, we note that the concentrations of salts can dramatically affect nuclear mechanics since divalent and, to a lesser extent, monovalent cations affect chromatin condensation (Aaronson, 1981; Dahl *et al.*, 2005). With changes in salt, nuclear volume changes significantly and may induce mechanical stress on the nuclear lamina. It is unclear exactly what effect these salt concentrations have on protein–protein interactions within the nucleus. Nuclei isolated in buffers with low salt tend to swell and resemble closely the contours and size of nuclei inside live cells. To accurately approximate nuclear mechanics inside the cell, it is usually desirable to mimic the intracellular ion concentrations, but the difficulty is that the ion concentrations inside the cytoplasm and within organelles have not been reproducibly determined, and values for ions such as calcium range from submillimolar to millimolar and can vary greatly as a function of disease (Dobi and Agoston, 1998). It is also difficult to predict the shift in nuclear salt homeostasis after isolation from the cell. Some studies have deliberately examined mechanics of isolated nuclei at extreme salt conditions to determine the maximum possible range of mechanical responses (Fig. 2). This strategy proves effective since the chromatin condensation and dilation appears to shift the load within the nucleus from the chromatin to the lamina (Dahl *et al.*, 2005).

V. Outlook

In outlining our methods used to understand the physical plasticity of nuclei in cell development and aging, we have focused in particular on lamin-A/C in the nuclear envelope. This is primarily because lamin-A/C is well known to undergo changes in normal stem cells thru aged cells, coupling to other epigenetic changes. What our physical measurements generally show is that nuclei flow and yield, like a plastic, beyond about 10 s of stress or strain, and that lamin-A/C at the envelope contributes in part to nuclear rigidification, as evident in a decreased creep compliance. It is attractive to think that a more flexible and fluid nucleus is a more functional nucleus.

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References

- Aaronson RP, Woo E. Organization in the cell-nucleus—divalent-cations modulate the distribution of condensed and diffuse chromatin. *J Cell Biol.* 1981; 90(1):181–186. [PubMed: 7251674]
- Andersen JS, et al. Nucleolar proteome dynamics. *Nature.* 2005; 433(7021):77–83. [PubMed: 15635413]
- Black BE, et al. An epigenetic mark generated by the incorporation of CENP-A into centromeric nucleosomes. *Proc Natl Acad Sci USA.* 2007; 104(12):5008–5013. [PubMed: 17360341]
- Caille N, et al. Contribution of the nucleus to the mechanical properties of endothelial cells. *J Biomech.* 2002; 35(2):177–187. [PubMed: 11784536]
- Castanotto D, Rossi JJ. The promises and pitfalls of RNA-interference-based therapeutics. *Nature.* 2009; 457(7228):426–433. [PubMed: 19158789]
- Constantinescu D, Gray HL, Sammak PJ, Schatten GP, Csoka AB. Lamin A/C expression is a marker of mouse and human embryonic stem cell differentiation. *Stem Cells.* 2006; 24:177–185. [PubMed: 16179429]
- Dahl KN, et al. Power-law rheology of isolated nuclei with deformation mapping of nuclear substructures. *Biophys J.* 2005; 89(4):2855–2864. [PubMed: 16055543]
- Dean DA, Kasamatsu H. Signal- and energy-dependent nuclear transport of SV40 Vp3 by isolated nuclei. Establishment of a filtration assay for nuclear protein import. *J Biol Chem.* 1994; 269(7):4910–4916. [PubMed: 8106464]
- Deguchi S, et al. Flow-induced hardening of endothelial nucleus as an intracellular stress-bearing organelle. *J Biomech.* 2005; 38(9):1751–1759. [PubMed: 16005465]
- Dobi A, Agoston D. Submillimolar levels of calcium regulates DNA structure at the dinucleotide repeat (TG/AC)_n. *Proc Natl Acad Sci USA.* 1998; 95:5981–5986. [PubMed: 9600903]
- Elbashir SM, Harborth J, et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature.* 2001; 411(6836):494–498. [PubMed: 11373684]
- Fire A, Xu SQ, et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature.* 1998; 391(6669):806–811. [PubMed: 9486653]
- Guilak F. The deformation behavior and viscoelastic properties of chondrocytes in articular cartilage. *Biorheology.* 2000; 37(1–2):27–44. [PubMed: 10912176]
- Johnson C, et al. Forced unfolding of proteins within cells. *Science.* 2007; 317(5838):663–666. [PubMed: 17673662]
- Lamond. 2009. <http://www.lamondlab.com/f7nucleolarprotocol.htm>
- Lieberman-Aiden E, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science.* 2009; 326(5950):289–293. [PubMed: 19815776]
- Pajerowski D, et al. Physical plasticity of the nucleus in stem cell differentiation. *Proceedings of the National Academy of Science - USA.* 2007; 104:15619–15624.
- Rowat AC, et al. Characterization of the elastic properties of the nuclear envelope. *J R Soc Interface.* 2005; 2(2):63–69. [PubMed: 16849165]
- Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. *Science.* 2006; 312(5776):1059–1063. [PubMed: 16645051]
- Wakayama T, Perry AC, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature.* 1998; 394(6691):369–374. [PubMed: 9690471]
- Wilmut I, Paterson L. Somatic cell nuclear transfer. *Oncol Res.* 2003; 13(6–10):303–307. [PubMed: 12725518]

Fig. 1.

Micropipette aspiration and deformation of nuclei. (A) Schematic showing the various substructures within the nucleus, including the nuclear lamin with lamin-A/C. (B) Aspiration at constant pressure of an hESC nucleus. The nucleus and cell flow slowly or “creep” into the micropipette. The cell contributes very little resistance, and based on many measurements of tissue fibroblasts, the elasticity prefactor E is about 5–10 kPa for the differentiated nuclei, and so the hESC nucleus is estimated to have a stiffness of about 1–2 kPa. (C) Aspiration of normal (“GM”) and lamin-A/C mutant (progerin, “AG”) fibroblasts that were latrunculin-treated to fluidize the actin cytoskeleton. Wild-type GFP-lamin-A/C is expressed in some aspirated nuclei to visualize the nuclear envelope and nucleoplasmic lamin structures as well as wrinkles and creases during aspiration. (D) The mutant cells were aged in culture (p denotes passage number) and exhibit rigidity associated with an accelerated aging phenotype. Rigidity is evident in creep compliance, including prefactor A , being lower for the aged nuclei.

Fig. 2. Mechanical responses. (A) Constant pressure effects a change in strain, and release of the stress leads to recovery except for a residual plastic strain. (B) Constant strain effects a change in stress, and release of the strain leads to recovery except for a residual plastic stress.