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Characterization of the elastic properties of the nuclear envelope

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Underlying the nuclear envelope (NE) of most eukaryotic cells is the nuclear lamina, a meshwork consisting largely of coiled-coil nuclear intermediate filament proteins that play a critical role in nuclear organization and gene expression, and are vital for the structural stability of the NE/nucleus. By confocal microscopy and micromanipulation of the NE in living cells and isolated nuclei, we show that the NE undergoes deformations without large-scale rupture and maintains structural stability when exposed to mechanical stress. In conjunction with image analysis, we have developed theory for a two-dimensional elastic material to quantify NE elastic behaviour. We show that the NE is elastic and exhibits characteristics of a continuous two-dimensional solid, including connections between lamins and the embedded nuclear pore complexes. Correlating models of NE lateral organization to the experimental findings indicates a heterogeneous lateral distribution of NE components on a mesoscopic scale.

Keywords: mechanics; confocal laser scanning microscopy; micropipette aspiration; green fluorescent protein-lamin A; nucleoporin p62

1. INTRODUCTION

Compartmentalizing the cell's genetic material, the nuclear envelope (NE) forms a boundary between the nucleoplasm and cytoplasm of eukaryotic cells and embeds nuclear pore complexes (NPCs) that help to regulate nuclear composition. From a mechanical perspective, the NE is a complex, composite structure consisting of NPCs spanning both inner and outer membranes (INM and ONM) that are separated by the nuclear lumen. Associated with the INM is the nuclear lamina, a filamentous structure consisting primarily of lamin type B (expressed in all cell types) and type A/C proteins (expressed only in differentiated cells) that interact with integral membrane proteins, chromatin (Mattout-Drubezki & Gruenbaum 2003; Zastrow *et al.* 2004), chromatin-binding proteins (Zastrow *et al.* 2004) and nuclear actin (Bettinger *et al.* 2004; Shumaker *et al.* 2003). The lamina's insolubility under harsh extraction conditions (Aebi *et al.* 1986; Moir *et al.* 2000; Aaronsen & Blobel 1975) and fragile nuclear phenotypes characteristic of laminopathic diseases (Lammerding *et al.* 2004; Mounkes *et al.* 2003; Goldman *et al.* 2002) suggest that the lamina provides the nucleus with structural integrity. Models of the lamina as a regular lattice network supporting the NE (Alberts *et al.* 2002; Lodish *et al.* 2001; Boal 2002) are based largely upon evidence of patches of regular lattice structure of the lamina in amphibian *Xenopus oocyte* NEs (Aebi *et al.* 1986), but to what extent this model reflects lamina structure in other cell types is unresolved. Indeed, evidence of a thick, non-homogeneous lamina structure in vertebrate cells (Fawcett 1966; Paddy *et al.* 1990), as well as clusters of an integral INM lamin receptor protein (LBR) (Makatsori 2004), suggests an *irregular*, laterally heterogeneous NE structure. Despite the obvious contribution of the NE to nuclear shape and structure, a physical understanding of how this material provides the nucleus with structural stability has not been described. A quantitative characterization of the NE's physical properties is necessary for an understanding of the mechanisms underlying mitosis, NE-related diseases and viral infection, as well as how the nucleus is protected and affected by external forces (i.e. mechanotransduction).

To achieve this, we have analysed NE deformations in living *HeLa* cells transiently expressing green fluorescent protein fused to lamin A (GFP-LamA) using an extension of fluorescence imaged microdeformation (FIMD) (Discher *et al.* 1994) to confocal imaged microdeformation (CIMD). Nuclei are also isolated from these cells to investigate *in vitro* behaviour of both GFP-LamA and the NPCs that are visualized by staining with an antibody directed against the nucleoporin p62. We have developed image-analysis techniques in conjunction with theory for a two-dimensional elastic material to quantify NE elastic behaviour and demonstrate the similar behaviour of the NE both *in vivo* and *in vitro*. Together with modelling of the mesoscopic structure of the lamina, we

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show that the NE is a material with a heterogeneous lateral organization that resists lateral shear forces and large-scale ruptures.

2. MATERIALS AND METHODS

2.1. Materials

All materials used were of the highest available quality and were obtained from the following sources: salts—Sigma (St Louis, USA); SYTOX Orange—Molecular Probes (Eugene, USA); Cy3-conjugated goat anti-mouse IgG—Jackson ImmunoResearch Laboratories, Inc. (West Grove, USA); p62 antinucleoporin IgG—BD Biosciences Pharmingen (San Diego, USA). Enhanced GFP fused to the amino terminus of the entire coding sequence for human prelamin A (GFP-LamA) was a kind gift from D. K. Shumaker and R. D. Goldman, Northwestern University, Chicago, USA (Moir *et al.* 2000).

2.2. Transfection

GFP-LamA amplified in Top10 cells and isolated using a Nucleobond AX plasmid purification protocol (Macherey-nagel, Duren) was transfected into subconfluent *HeLa* cells together with twofold excess carrier DNA (Herring testes DNA, Clontech, San Jose, USA) using $CaPO_4$ (Invitrogen, Carlsbad, USA).

2.3. Nuclear isolation

Nuclei were isolated from *HeLa* cells as previously described (Muramatsu *et al.* 1963). After centrifugation of the dounced nuclei, the supernatant was adjusted to have a final 'physiological' concentration (Jackson *et al.* 1988) of 130 mM KCl, 1.5 mM $MgCl_2$, 10 mM Na_2HPO_4, 1 mM Na_2ATP and 1 mM DTT, pH 7.4, in which the nuclear pellet was resuspended. All steps subsequent to trypsinization were carried out at 4 °C.

2.4. Confocal imaged microdeformation

Pipettes were pulled from glass capillary tubes (World Precision Instruments, Sarasota, USA) using a pipette puller (Sutter Instruments Co., USA) and forged (Microforge MF-900, Narishige, Japan) to ensure a flat pipette tip. The final inner diameter of the pipette was typically $2R_p = 2.5$–5.0 µm. Micropipettes were treated with silanization fluid (Number II, Sigma) and dried in an oven at 90 °C for about 2 h prior to aspiration to prevent adhesion of nuclei/cells to the glass surface. The micropipette was mounted on a Zeiss Axiovert 200M microscope equipped with a LSM 510 laser scanning module. Pressures were applied to nuclei through the micropipette using a custom built manometer system (Evans & Needham 1987). After applying pressure to interphase nuclei (identified as non-dividing, single, morphologically intact nuclei), the tongue length, L, stabilizes before acquiring an image. The pressure was incrementally increased in this manner, and nuclei were visualized. eGFP was excited using an argon laser (488 nm), and Cy3 using a He–Ne laser (543 nm). Multichannel acquisition was used to acquire images with more than one fluorophore. The pinhole size was typically set to approximately 1 Airy unit, resulting in a confocal slice thickness (z-resolution) of approximately 1 µm. *In vitro* nuclei were visualized in a two-well #1.0 borosilicate chamber (Nalge Nunc Int.) at room temperature (approximately 25 °C). For *in vivo* experiments, cells were grown on coverslips that were transferred to the viewing chamber at the time of the experiment and immersed in a CO_2-independent medium (minimum essential medium without phenol red containing 10% foetal bovine serum, 1% glutamate, 1% sodium pyruvate, 1% penicillin/streptomycin and 1% 1 M HEPES (Invitrogen)) at 37 °C. Three-dimensional reconstructions were generated using LSM 510 v. 3.0 software.

2.5. Modelling of intensity profiles

We take the thin elastic shell as a model for the NE and assume that the in-plane strain and stress can be described by a two-dimensional elastic model. Opposite to the situation in a fluid, the nearest neighbour structure of the NE is fixed, i.e. the molecular constituents are fixed relative to each other (characteristic of a solid), so for the purpose of modelling the surface is divided into elements, each of which contain some fixed surface components. In the equilibrium (undeformed) state, the characteristic length of an element is l_0. For a surface of revolution, such as the aspirated tongue of the NE in a micropipette, the elements along the meridian and latitude are labelled with the dimensions l_m and l_ϕ. We can write an approximate free energy density per element as (Ogden 1984; Stokke *et al.* 1986)

$$f = \frac{K}{2}\left(\frac{l_m l_\phi - l_0^2}{l_0^2}\right)^2 + \frac{\mu}{2}\left(\frac{l_m - l_\phi}{l_0}\right)^2. \qquad (2.1)$$

Here K and μ are the compression and shear moduli with units of J m^{-2}. Analysis of equation (2.1) shows that under micropipette deformation the density distribution in the cylindrical part of the shell is expected to decline exponentially throughout the pipette (along the x-axis) with the form $\rho(x) \propto \exp(-ax/R_p)$. This form holds if the NE behaves as a Hookean elastic sheet with $0 < a \leq 2$, where $a = 2$ for a simple homogeneous elastomer (Markin & Kozlov 1988). Fitting this expression to the experimental intensity profile data yields a value of a, a parameter that depends only on K/μ and the densities in the tongue tip and the bulk nucleus.

2.6. Modelling of intensity distributions

The observed pixel intensity distributions reflect the density and distribution of lamin proteins on a mesoscopic scale. Analysis and comparison of the mean intensity and variance to the predicted model behaviour provide insights into the underlying lamina structure. Details can be found in the Electronic Appendix.

3. RESULTS AND DISCUSSION

3.1. In vivo *nuclei*

In the initial state, the fluorescence intensity of GFP-LamA nuclei is localized primarily at the peripheral rim of the nuclei (inner nuclear membrane; figure 1*b*,*d*,*e*). Owing to the transient nature of the transfection, varying levels of GFP-LamA expression were observed, so only nuclei expressing medium levels of the construct (an intense rim and faint nucleoplasmic staining) were chosen for aspiration. A micropipette (inner radius $R_p \sim 1.5$–2.0 μm) was used to aspirate the nucleus through the plasma membrane of a living cell, and deformations of the nucleus were monitored by acquiring a series of confocal fluorescence images through the axial dimension in order to reconstruct a three-dimensional image. Under these conditions, the nuclear membrane and GFP-LamA-delineated lamina were both aspirated into the pipette (figure 1*i*). The fluorescence intensity is not homogenous throughout the extension in the pipette (tongue), but rather a gradient in fluorescence intensity is observed (figure 1*j*). As the fluorescence intensity (I) reflects the density of GFP-LamA (ρ), the observed tongue gradient signals the dilation of the lamins in the tip of the tongue, while the increased intensity about the mouth of the pipette indicates a local increase in the density (compression) of GFP-LamA. Although faint at the tongue tip, the GFP-LamA intensity is seen to coincide with the INM (figure 1*i*), indicating that the lamina remains associated with the NE despite the perturbation. The pixel intensity varies in the cylindrical tongue up to the edge of the hemispherical cap. The exponential form $\rho(x) \propto \exp(-ax/R_p)$ can be fit to this pixel intensity profile, where x is the position along the tongue (figure 1*j*,*k*). Our results for *in vivo* nuclei yield a small variation in a-values, $a = 0.41 \pm 0.04$ ($n = 7$)[1]. Together with the observed intensity ratio, $\langle I_i \rangle / \langle I_o \rangle = 0.5 \pm 0.1$, between the intensities in the aspirated tip (I_i) and bulk nucleus (I_o), this corresponds to an elastic moduli ratio, $K/\mu = 1.5 \pm 0.3$, where K is the expansion modulus and μ the shear modulus for a two-dimensional isotropic elastic material. From this deformational analysis, we conclude that the NE is a two-dimensional elastic material that resists shear forces like a solid.

The persistence of the intensity gradient over time (30–45 min) indicates that the structure of the underlying aspirated NE with which GFP-LamA is associated, remains constant. This observation is consistent with fluorescence recovery after photobleaching analyses that revealed slow recovery of GFP-lamin fusions in interphase cells (Daigle *et al.* 2001; Moir *et al.* 2000).

3.2. In vitro *nuclei*

Interpreting the aspiration of nuclei in whole cells is complicated by the presence of the cytoskeleton as well as various membranes and organelles. For this reason, isolated nuclei provide a much cleaner system in which other structural components, such as NPCs, can be indirectly labelled in order to further investigate