

## Supporting Text

**Orientalional Ordering of Filaments in the Lamina.** The birefringence seen in Hutchinson-Gilford progeria syndrome (HGPS) nuclei could be due to stress in the lamina or formation of orientationally ordered phases of lamin filaments. Stress in a material can cause a change in the index of refraction along the directions of the stresses, resulting in optical birefringence. However, isolated HGPS and WT nuclei were examined for birefringence under the same buffer and adhesion conditions, so it is unlikely that there are any significant differences in the stresses between the HGPS or WT laminae. To determine whether the HGPS birefringence could be due to lamin filament alignment, we calculated the theoretical volume fraction required for the isotropic-nematic transition for lamin filaments and compared it with the approximate volume fractions of lamins in the WT and HGPS laminae.

The lamin filaments of the present problem are rod-like molecules with a length ( $L$ ) of  $\approx 52$  nm and a diameter ( $D$ ) of  $\approx 10$  nm (1). The transition volume fraction for isotropic to nematic, smectic, and orientationally ordered solid phases has been theoretically calculated by using density functional theory as a function of the aspect ratio  $L/D$  (2). For  $L/D \approx 5$ , the aspect ratio of lamin filaments, the transition from isotropic (I) to nematic (N) phase occurs at  $\phi_{I-N} \approx 0.4$  (2). A transition to the layered and aligned smectic phase occurs for  $\phi > 0.5$ , and an orientationally ordered solid phase forms for  $\phi > 0.7$ . As an example, cellulose rods with  $L/D \approx 5$  have an experimental isotropic to nematic transition at  $\phi_{I-N} \approx 0.4$  (3).

We have roughly estimated the volume fraction of filaments in the WT and HGPS nuclear lamina from various literature sources and our own data. We estimate the diameter of the nucleus to be  $10 \mu\text{m}$  (from Figs. 1, 5, and 8) and use a previously determined lamina thickness of  $60$  nm (4); thus, the volume of the lamina shell is  $V_{\text{lamina}} = 18 \mu\text{m}^3$ . Using values of  $10^6$  of each of lamins A, B, and C per nucleus (5) and the relative concentrations of lamins in the lamina that we determined by immunofluorescence, we can derive an approximate (“effective”) volume fraction ( $\phi_{\text{eff}}$ )

of lamins in the lamina. In WT nuclei, 80% of lamin B and 40% each of lamins A and C are associated with the nuclear envelope at any given time.

$$\phi_{eff} = \frac{nV_{filament}}{V_{lamina}}. \quad [1]$$

Here the number of filaments is  $n \approx 1.5 \times 10^6$ , and the volume of the individual lamin filament, assumed to be a cylinder, is  $V_{filament} = \pi \times (d/2)^2 \times L$ . For a WT nucleus,  $\phi_{eff} = 0.34$ , which is below  $\phi_{I-N}$ . If we increase the number of lamin filaments at the envelope so that 2/3 of lamins are in the lamina (80% of lamin B, 80% lamin A, and 40% lamin C), then  $\phi_{eff}$  increases to 0.45 and, hence, is larger than  $\phi_{I-N}$ . As more of the lamins enter the lamina, more tightly packed phases also could occur such as the smectic or orientationally ordered solid phases.

Of course, we have made several physical assumptions about our system both in adopting the theory above and in choosing filament number densities. For example, we have assumed that the lamin interactions are like those of rigid rods. If lamins experience other interactions, for example due to van der Waals forces, then the volume fraction for formation of nematic, smectic, or orientational solid phases could be different (6). It is interesting, nevertheless, that the estimated filament volume fractions in the nuclear envelope are so close to  $\phi_{I-N}$ . Although refinement of this theory and numerical analysis is a topic for further study, clearly the increase in lamin filament concentration in the HGPS lamina has potential to induce ordered phases.

We also did not notice any change in the birefringent intensity when the HGPS nucleus was rotated between the crossed polarizers, suggesting an absence of global lamin orientation. This constant and bright birefringent pattern could result from a 3D stacking of ordered phases. It is more likely, however, that the lamin filaments have assembled into many small, uncorrelated but orientationally ordered microdomains, each large enough to exhibit birefringence.

## Methods

**Cell Culture and Transfection.** Cells were grown in minimum essential medium (Invitrogen, no. 10370-021) supplemented with 15% FCS, 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin at 37°C in 5% CO<sub>2</sub>. HeLa cells were grown in DMEM supplemented with 10% FCS.

Cells were electroporated with a BTX electroporator ECM 830 by using 5 µg of plasmid DNA and 15 µg of sheared salmon sperm carrier DNA in a 2-mm gap cuvette. The electroporation settings were 175 V, 1-ms pulse, four pulses, and 0.5-s interval between pulses for primary fibroblasts and 225 V, 1-ms pulse, four pulses, and 0.5 s interval for HeLa cells.

For dilation/compression experiments with transfected HeLa cells, HeLa cells were transfected with PolyFect (Invitrogen) transfection reagent as per manufacturers instructions.

**Generation of pEGFP-Δ50 by PCR.** Two pairs of primers were used, two external to the deleted region (Δ50ext-for: 5'-cagcctgcgtacggctctc-3' and Δ50ext-rev: 5'-gatcagttatctagatccgg-3', containing, respectively, BsiWI and XbaI restriction sites), and two antiparallel internal primers, spanning the deleted region (Δ50int-for: 5'-cccagagccccagaactgcagc-3' and Δ50int-rev: 5'-gggctctgggctcctgagccg-3'). In the first step, two regions flanking the deleted sequence were amplified by using the primer pairs Δ50ext-for/Δ50int-rev and Δ50int-for/Δ50ext-rev. The two amplification products, partially complementary to each other, were mixed and used in the second PCR reaction in the presence of the external primers. The final PCR product was cloned into the BsiWI/XbaI site of pEGFP-lamin A. Sequences were confirmed by double-stranded sequencing.

**Western Blotting.** For Western analysis, ≈5 million cells were collected, washed in PBS, and resuspended in Laemmli buffer. Equal amounts of the protein samples were loaded

on 7.5% or 4–20% Tris-glycine mini gels (Bio-Rad). The proteins were blotted on an Immobilon P membrane (Millipore) by using a tankblot system (Bio-Rad) in 25 mM Tris, pH 7.5/0.192 M glycine/20% methanol at 4°C for 2 h at 300 mA. After blocking in 5% skim milk/TBST (20 mM Tris, pH 7.5/137 mM NaCl/0.1% Tween 20) for 1 h, the blots were probed with the indicated antibodies. The blots were developed by using the ECL system (Amersham Pharmacia Biotech).

**Measuring Nuclear Swelling and Shrinking.** Swelling and micropipette aspiration (MPA) images were acquired on an IX81 Olympus inverted microscope with a  $\times 60$  (oil, 1.45 NA) objective and collected by a CoolSnap camera (Photometrics) by using Metamorph (Molecular Devices) WTs. Lengths were measured by using IMAGE J (National Institutes of Health). Nuclear sizes were measured by two methods for speed and accuracy. For most measurements, nuclei were labeled with DAPI, fluorescently imaged, and fluorescent pixels were counted by using IMAGE J. To ensure accuracy, some nuclear areas were measured manually in IMAGE J by tracing around the nuclear envelope of the brightfield image.

**Micropipette Aspiration.** Capillary tubes of 1.0 mm inner diameter (ID; World Precision Instruments) were pulled into micropipettes. Smaller ID micropipettes ( $R_p$  2–4  $\mu\text{m}$ ) were produced by using a Flaming-Brown Micropipette Puller (Sutter Instrument, Novato, CA) and cut to various diameters by using a deFonbrune-type microforge (Vibratome, St. Louis). For larger ID pipettes ( $R_p = 3\text{--}5$   $\mu\text{m}$ ), a PMP102 micropipette puller (MicroData Instrument, South Plainfield, NJ) was used, and no forging was required. Micropipettes were attached to a water reservoir. Before nuclear aspiration, large volumes (50–100  $\mu\text{l}$ ) of the appropriate buffer were aspirated into the micropipette.

Suction was applied by a syringe, and the pressure was measured by a mercury U tube manometer (Fisher Scientific). Pressures for different experiments ranged from 2.9 kPa to 16.5 kPa. Isolated nuclei were aspirated into micropipettes with a set, step aspiration pressure  $P$ , and the extension into the pipette  $\Delta L$  into the pipette was followed from 0.2 s to 500 s on a log time scale. As in previous studies, the creep compliance  $J$  was

calculated from  $\Delta L$ ,  $P$ , the pipette radius  $R_p$ , and numerical prefactor, which depends on the pipette thickness (7, 8).

$$J(t) = \frac{1.4\pi\Delta L(t)}{R_p P}. \quad [2]$$

Creep compliances were converted to elastic moduli by (9).

$$E(t) = \frac{\sin(\alpha\pi)}{\alpha\pi} \frac{1}{J(t)}. \quad [3]$$

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