**Nuclear mechanics**

**Lamin webs and pathological blebs**

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Moreover, as with the cytoplasm there are a number of pathologies associated with altered nuclear mechanics. However, elucidating the role of mechanics requires new approaches in the nucleus, due to its size, geometry and unique structural features.

The nucleus typically contains no microtubules, and while actin is present, its form is poorly understood. Indeed, despite recent advances in imaging actin within living nuclei,7 there is still little known about its potential mechanical role. Instead, one of the key mechanical components of nuclei are the nuclear lamins, members of the Type V intermediate filament protein superfamily. The four major lamins can be grouped into A and B types. A type lamins A (LA) and C (LC) are encoded by the same gene, while B type lamins B1 (LB1) and B2 (LB2), are coded by different genes.8

Nuclear lamins form a dense meshwork organized in a thin shell curving along the inner membrane of the nuclear envelope. This lamina has a thickness of only 10–80 nm, several orders of magnitude smaller than the typical nuclear radius 5–10 μm. Fluorescence recovery after photobleaching (FRAP) experiments indicated that while nucleoplasmic lamins are dynamic, lamins within the cortex may be largely stable and immobile.12 This observation suggests that the integrity of the lamin meshwork is stabilized by filament crosslinking, although little is known about the way in which lamin-associated proteins regulate the structure of the lamina.13 Interestingly, reconstituted lamin networks—in the absence of

A normal in the three-dimensional shape of the nucleus are associated with a number of genetic diseases. These shape distortions include lobulated structures, with localized bulges referred to as nuclear blebs. Blebbing can result from mutations in genes encoding lamin intermediate filaments that form the lamin cortex, a thin meshwork lining the nuclear envelope. However, the biophysical origins of nuclear blebs remain a mystery. A recent study by Funkhouser et al. provides a theoretical model in which the lamin cortex is modeled as a thin, inhomogeneous elastic shell. This model shows that partial segregation of different lamin sub-networks—each with distinct mechanical properties—can lead to shell morphologies similar to blebbed nuclei in living cells.

The mechanical properties of cells are intimately tied to their biological function. The dependence of biological function on mechanics is now well established,1 including decisions as important as whether a cell should grow or divide,2 where to migrate,3 or how stem cells should differentiate.4 The importance of mechanics has been increasingly appreciated over the last decade. Indeed, mechanical forces are associated with chromatin remodeling and modulation of transcriptional activity.5-7

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auxiliary proteins—all were found to form predominantly elastic gels, which stiffen strongly under strain and exhibit a large mechanical resilience; these observations suggest substantial interactions that prevent inter-filament sliding. Other in vitro experiments on various IFs, including neurofilaments and vimentin, suggest that divalent ions can facilitate crosslinks preventing inter-filament sliding. Other in vitro experiments on various IFs, including neurofilaments and vimentin, suggest that divalent ions can facilitate crosslinks between filaments, indicating that electrostatic interactions could contribute to IF network stability. Although much remains to be understood, the lamin meshwork appears to form a relatively elastic solid-like cortical shell, which provides mechanical support to the nucleus. Consistent with the idea that the lamin cortex is an important structural component, various anomalies in the three-dimensional shape of the nucleus are caused by disease-associated mutations in genes encoding for the basic constituents of the lamina meshwork (for a recent review, see ref. 17). While a normal nucleus typically exhibits a highly smooth, rounded shape, such mutations can give rise to severely mis-shapen nuclei; for example, cell nuclei from patients with Hutchinson-Gilford progeria syndrome exhibit highly lobulated shapes, with localized bulges typically referred to as “nuclear blebs.” These pathological nuclear shape distortions often result from changes in the elasticity of the lamin shell. Nuclear blebbing could thus be related to mechanical instabilities of thin shells, well-known from elasticity theory. A key parameter in the analysis of such shells is the ratio of the shell thickness, h, to its radius, R; for thin shells h/R << 1, which has important consequences for the resulting behavior. In particular, the two dimensional deformation energy associated with stretching scales with h/R, while the bending energy scales with (h/R)^3. Although elastic shells can undergo both stretching and bending deformations, thin shells are much more compliant to bending than to stretching, since (h/R)^3 << h/R. This simple fact has myriad implications for the three dimensional structures of deformed shells, from the crumpling of dehydrated pollen grains, to the indentation of viruses under a localized stress.

An important potential difference between the nuclear cortex and a simple elastic shell is that the nuclear lamina is a composite material; this could be relevant, because material inhomogeneities are known to have important implications for elastic instabilities. Lamins LA and LBI can interact with each other both homotypically and heterotypically, but there is evidence that A- and B-lamins form separate, but interconnected networks. Moreover, imaging reveals regions that appear to be altered, with some partial overlap. It has also been suggested that B1-type lamins (LBI) play an important role in organizing the A- and B-type microdomains. For example, in LBI silenced cells, larger islands of A-type domains were observed and the meshsize of LB2 and A/C regions appeared to be enlarged. Interestingly, blebs were found to be enriched with A-type lamins and lacking in B-type lamins (Fig. 1A), suggesting that segregation between the A- and B-type networks could lie at the origin of blebbing. Motivated by these observations, a recent computational study by Funkhouser et al. proposed a two-component continuum elastic shell model for the lamina. Each component is postulated to correspond to either of the structurally dissimilar A or B-type lamins, and thus, each component is allowed to have its own distinct mechanical and morphological properties. The model of Funkhouser et al. incorporates a reference shape (undeformed state) of the lamina as a sphere with a radius of curvature R. Observations suggest that the lamin density in A-enriched blebbed regions appears to be reduced, which could be caused by the blebbing event itself. Alternately, it could be an intrinsic property of the A-type subnetwork. The model of Funkhouser et al. assumes the latter, imposing a larger radius of curvature for A-type regions R_A > R, and a larger meshsize (average spacing between filaments). Thus, the two components of the model shell not only have distinct elastic properties, but also distinct preferred curved geometries. Funkhouser et al. used a finite element model to describe this heterogeneous curved elastic system. Vertices are
distributed randomly over a 3D surface with a spherical topology, while maintaining a fixed distance between the vertices. Vertices are either A- or B-type lamins, representing a region enriched with the respective lamin type networks. To capture the stretching elasticity of the shell, vertices are connected by A- or B-type springs with a distinct stiffness and rest length. Bending contributions are captured by an energy term that penalizes departures from the preferred curvature between the triangle elements belonging to the apex of a vertex. This constitutes a minimal numerical model to describe an isolated, heterogeneous elastic shell in the absence of pressures or tensions.

Starting with a fraction f of B-type nodes randomly mixed with A-type nodes, Funkhouser et al. employ a Monte Carlo annealing procedure in which A and B nodes are switched to relax the node distribution as the temperature is effectively reduced to zero (quenched exponentially) through a sequence of equalizations. This procedure aims to find the distribution of A- and B-type nodes that minimizes the elastic energy. Even though there is no penalty (or gain) for mixing of A and B type elements in their model, they find that the shell segregates into large A and B type domains. The authors report that this segregation depends only on the difference in preferred curvature between the two types of elements (which can be tuned by varying a scaling factor of the metric tensor $M_i$, for A-type nodes, holding $M_j = 1$). Such a segregation is energetically favorable since it reduces the amount of interfacial energy between A and B regions where large deformations would be necessitated by their different preferred curvatures. The segregated two-component shells have striking morphologies: For large differences in preferred curvature ($M_i > 2$), the shell has a wrinkled structure for B-type fractions $f < 0.5$. This wrinkling hints toward an elastic instability caused by the constraints imposed on the expanding A-regions by the surrounding B-regions. By contrast, for higher B-type fractions $f > 0.5$, A-type regions form smooth cap-like protrusions, resembling most closely typical morphologies of nuclear blebs, as shown in Figure 1.

As pointed out by the authors, the results of their Monte Carlo procedure may depend on the details of the path toward the lower energy states. In particular, when the computational system is cooled down too fast, the distribution of A and B components will essentially freeze into a certain state of (partial) segregation. The simulated shell morphologies thus do not necessarily represent global energy minima, but rather an energy minimization with a partially relaxed distribution of A and B regions. Indeed, when the simulations are relaxed at a lower rate, fewer, but larger blebs form. Clearly, the state with the lowest elastic energy in this model is obtained when the A and B regions completely demix as to obtain two large domains separated by a single interface.

A completely demixing is not typically observed in cells. Funkhouser et al. speculate that this could be due to the limited mobility and turnover of filaments in the blebs. It is certainly possible that significant energy barriers prevent the system from relaxing to its fully segregated ground state. It is puzzling though that the system seems sufficiently dynamic to reach this partially demixed state, but then would appear to arrest completely. Funkhouser et al. suggest that a model that also includes the viscoelastic nature of the lamina could explain this behavior. Relaxation rates could effectively vanish as the segregated domains become larger, preventing the system from reaching equilibrium on physiological time scales. It would thus be interesting to consider models that include aspects of the reorganization kinetics of the nuclear lamina, although the viscoelastic properties of the lamina in living cells are unknown.

The segregation driven by differences in the elasticity of the lamin sub-networks proposed by Funkhouser et al. provides an interesting perspective on the biophysical origins of nuclear blebs. But it is important to recognize that the model contains a range of geometrical and mechanical parameters which are not well-known under in vivo conditions, and it remains unclear whether such a mechanism is actually at play within living cells. Qualitatively different models are conceivable. For example, it may be possible that the lamina itself is not the driving force for the formation of blebs. Instead, local defects in the lamina could destabilize the laminar shape against other forces, such as any osmotic pressure difference between the nucleus and the cytoplasm, or stresses arising from the high density of compacted chromatin. Indeed, a recent study observed nuclear blebbing in mechanically compressed tissue culture cells and nuclear blebbing has recently been shown to be a pathway of mRNA export, demonstrating that blebs can occur in cells in the absence of lamin mutations. Moreover, a more complete, thermodynamic model would also consider the entropy and energy of mixing of different lamin sub-networks, both of which could counteract the tendency toward a demixed state. It is also possible that coupling between the lamina and the cytoplasm plays an important role in stabilizing the shape of the lamina and the segregation dynamics of the lamins. An analogous arrest of a demixing phase separation of lipid domains may arise from coupling between the cell membrane and underlying actin cortex. Future work will investigate how these mechanical and structural effects could give rise to the cell and organism level phenotypes observed in diseases such as Hutchinson-Gilford progeria and Emery-Dreyfus muscular dystrophy.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

Acknowledgments

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