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REVIEW

SUBJECT COLLECTION: EXPLORING THE NUCLEUS

Mechanics of nuclear membranes

Ashutosh Agrawal[1,*] and Tanmay P. Lele[2,*]

ABSTRACT

Cellular nuclei are bound by two uniformly separated lipid membranes that are fused with each other at numerous donut-shaped pores. These membranes are structurally supported by an array of distinct proteins with distinct mechanical functions. As a result, the nuclear envelope possesses unique mechanical properties, which enables it to resist cytoskeletal forces. Here, we review studies that are beginning to provide quantitative insights into nuclear membrane mechanics. We discuss how the mechanical properties of the fused nuclear membranes mediate their response to mechanical forces exerted on the nucleus and how structural reinforcement by different nuclear proteins protects the nuclear membranes against rupture. We also highlight some open questions in nuclear envelope mechanics, and discuss their relevance in the context of health and disease.

KEY WORDS: LINC complex, Membrane mechanics, Nuclear envelope

Introduction

The nuclear envelope partitions nuclear contents from the cytoplasm and controls access of cytoplasmic proteins to the genome (Hetzer, 2010; Ungricht and Kutay, 2017). It comprises the outer and inner nuclear membranes (ONM and INM, respectively), which are both lipid bilayers. The nuclear envelope has remarkable geometric features (Fig. 1). The two membranes are separated by a fairly regular distance of ∼30 to 50 nm (Franke et al., 1981). These membranes are fused at hundreds of sites, which are almost uniformly distributed over the membrane surface. At these sites, the membranes undergo extreme bending and form a donut-like structure (Watson, 1955). The donut-shaped pores in the membranes house three-dimensional protein channels called nuclear pore complexes that regulate the active transport of macromolecules into and out of the nucleus. In some cell types, the nuclear envelope displays extreme bending during inward invaginations into the nucleoplasm, with the resulting structure called the nucleoplasmic reticulum (NR) (Malhas et al., 2011). Under certain conditions, the nuclear membrane can form blebs, which can rupture, and allow diffusive mixing of nucleoplasmic and cytoplasmic contents.

The nuclear envelope is subjected to forces originating in the F-actin and microtubule cytoskeleton (Davidson et al., 2014; Fridolfsson and Starr, 2010; Lele et al., 2018; Luxton et al., 2011; Roman and Gomes, 2017; Sims et al., 1992; Toh et al., 2015; Wilson and Holzbaur, 2012, 2015; Zhu et al., 2017). Nesprin family proteins in the ONM, which contain the Klarsicht/ANC-1/SYNE homology (KASH) domain, connect with cytoskeletal motors and/or cytoskeletal elements, which transfer tensile and shear force across the nuclear envelope through linkages between the nesprin KASH domain and INM Sad1/UNC-84 (SUN) proteins (Fig. 2). The SUN–KASH complex is called the linker of nucleoskeleton and cytoskeleton (LINC) complex (Crisp et al., 2006; Majumder et al., 2019; Starr and Fridolfsson, 2010). The nuclear lamina underlies the nuclear membrane in most mammalian cell types and is composed of the nuclear lamins. In these cell types, the nuclear lamina may balance a portion of forces transmitted by the LINC complex and mediate the mechanical response of the nucleus to these forces (Dahl et al., 2004; Davidson and Lammerding, 2014). In addition, differences in the hydrostatic pressure between the nucleoplasm and the perinuclear space can act on the INM, and the pressure difference between the perinuclear space and the cytoplasm can act on the ONM.

This complex architecture of the nuclear envelope means that the response of the nuclear membranes to the mechanical forces it experiences cannot be predicted simply from a knowledge of the mechanical properties of single membranes, which have been extensively studied *in vitro* (Boal and Boal, 2012; Phillips et al., 2012). Likewise, the mechanical properties of the nuclear membranes are likely to be very different from those of the plasma membrane, which is a single bilayer. In this Review, we first discuss how lipid bilayer bending and stretching might determine its response to mechanical force, before discussing how the structure of the nuclear membrane and its rupture behavior can be understood by considering the mechanical properties of the bilayers. Throughout, we identify open questions that may be answered by examining the mechanics of nuclear membranes. Given the many recent reviews on the mechanics of the nucleus and the force exerted on it by the cytoskeleton (Jahed and Mofrad, 2019; Kirby and Lammerding, 2018; Lele et al., 2018; Maurer and Lammerding, 2019; Uhler and Shivashankar, 2018; Zhu et al., 2017), we do not discuss these aspects except in the context of nuclear membrane mechanics.

Energetics of lipid membrane deformation

Lipid bilayers are ∼4–5 nm thick, but as their width can extend over several microns, individual nuclear membranes can be mechanically modeled as 2D surfaces (Boal and Boal, 2012). The elastic deformation response of these membranes to mechanical force is primarily due to their resistance to bending and stretching. Membrane deformations modulate the separation of lipid headgroups, which can expose hydrophobic lipid tails to the surrounding aqueous medium. Such changes in the relative orientation (bending) or the relative distance between the lipids (stretching) costs energy and results in the resistance of an elastic membrane to bending and stretching deformations (Boal and Boal, 2012).

However, unlike what is seen in purely solid-like 2D materials (e.g. rubber balloons), lipids also continuously diffuse on the nuclear membrane surface and also exchange with the endoplasmic reticulum (ER). This lipid transport imparts a viscous fluid-like behavior to lipid membranes, which helps them to resist mechanical shear stresses (Arroyo and Desimone, 2009; Rangamani et al., 2013). Thus, lipid membranes are both 2D fluid and 2D elastic materials.

The equilibrium shapes of lipid membranes under mechanical stresses can be computed by using appropriate models that calculate

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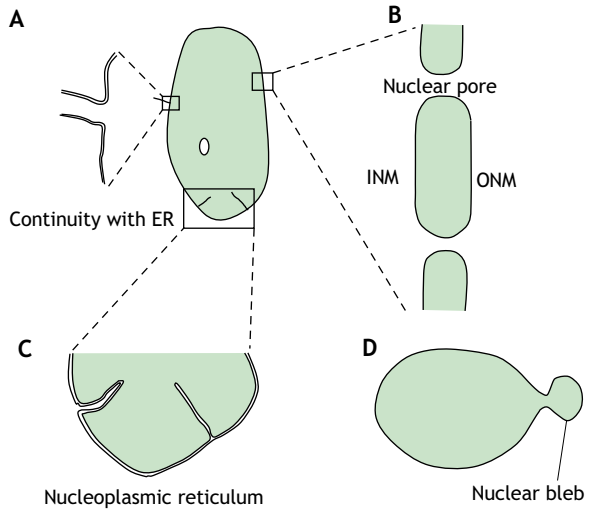

Fig. 1. Diversity of nuclear membrane shapes. (A) Nuclear membranes are continuous with the ER membranes. (B) The inset shows the ONM fused with the INM in a donut-like geometry at the nuclear pore. (C) The inset shows the NR; here, either the INM alone or both the INM and ONM can bend inwards. (D) The nuclear membrane can bleb, ultimately resulting in membrane rupture.

their minimum energy configurations (Boal and Boal, 2012; Phillips et al., 2012). The energy of the membrane is primarily based on two contributions: the energy of bending and the energy of areal stretch. Both these energies have to be accounted for when computing membrane shapes.

The energy required to locally bend the lipid membrane depends on two curvatures, the mean curvature H and the Gaussian curvature K (Canham, 1970; Helfrich, 1973). The mean curvature is calculated as the average of the minimum and maximum curvatures (principal curvatures) at any point on the nuclear membrane surface, while the Gaussian curvature is calculated as the product of the two. The popular Helfrich–Canham model (Canham, 1970; Helfrich, 1973) accounts for these two curvatures. In this model, the bending energy per unit surface area of the membrane (W_b) is given by:

$$W_b = \frac{\kappa(2H - H_0)^2}{2} + \bar{\kappa}K, \quad (1)$$

where κ and $\bar{\kappa}$ are bending moduli, and H_0 is the preferred curvature. These parameters depend on the composition of the lipid membranes. For the majority of lipids, membranes have a κ of ~20 k_BT and $\bar{\kappa}$ is around −20 k_BT (Hu et al., 2012), where k_B is the Boltzmann's constant and T is absolute temperature. The preferred curvature H_0 is determined by the shape and the composition of lipids. Lipids in bilayers can have cylindrical or non-cylindrical (cone-shaped or inverted cone-shaped) shapes (Fig. 3A). Bilayers with an asymmetric distribution of non-cylindrical lipids in the two leaflets will possess a preferred curvature. Bilayers with higher cone-shaped lipid content in the upper leaflet will bend upwards, while they will bend downwards when the lower leaflet is enriched in cone-shaped lipids (Fig. 3B). The energy required to locally stretch lipid membranes (W_s) is proportional to the dimensionless areal stretch ϕ and is given by:

$$W_s = \kappa_a \frac{\phi^2}{2}, \quad (2)$$

where κ_a is the stretch modulus. For lipid membranes, κ_a is ~55–70 k_BT/nm^2 or 230–290 mN/m (Phillips et al., 2012). The product of the stretch modulus and the areal stretch yields the tension in the membrane. Spatial variations in the composition of the lipids and/or protein concentration in the membrane can cause spatial variations in membrane tension (Agrawal and Steigmann, 2009; Shi et al., 2018).

If the areal stretch modulus and the bending rigidity of lipid membranes is known, it is possible to calculate their equilibrium shapes under defined geometric constraints (boundary conditions), and under known applied mechanical stresses. This approach has been used to calculate equilibrium shapes of the nucleus and nuclear membranes (Lim et al., 2007; Noguchi, 2016; Torbati et al., 2016), which is discussed in the next section. This approach has also been extensively used to predict the shapes of a diverse array of other lipid membrane structures, such as the membranes of

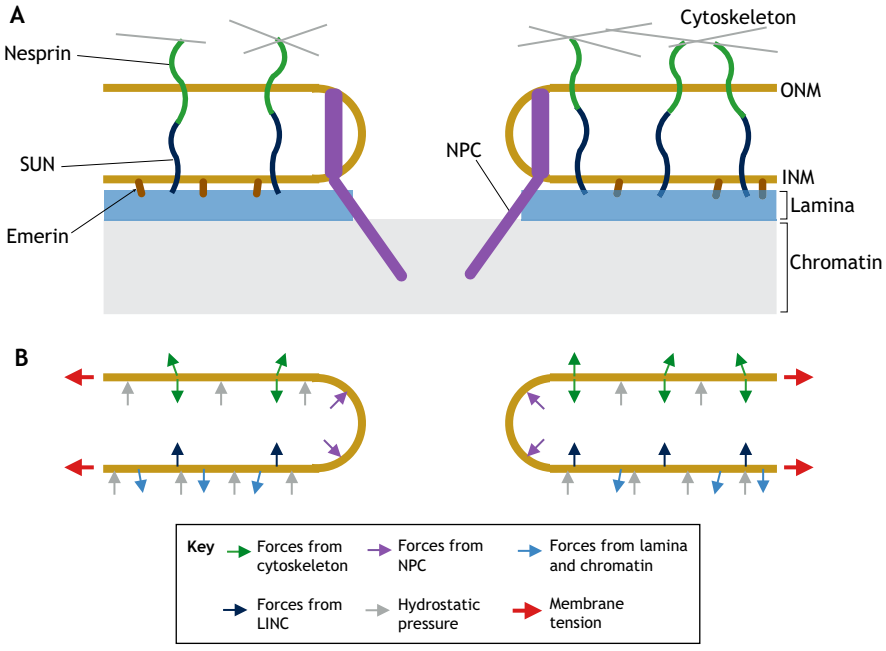

Fig. 2. Schematic illustration of the nuclear envelope components that bear mechanical loads. (A) ONM nesprins bind to INM SUN protein trimers (Sosa et al., 2012), creating a mechanical linkage between the ONM and the INM; ONM nesprins link to the cytoskeleton, while INM proteins like SUN and emerin link the INM to the nuclear lamina. (B) Diagram depicting the possible forces on the ONM and INM. Tensile and shear forces from the cytoskeleton can act on the ONM, and forces from the lamina and/or chromatin can act on the INM. These forces are resisted in part by the inwardly directed forces in the LINC complex proteins. As the membrane passes through the NPC, the NPC can apply forces on the membrane to prevent an expansion of the NPC radius. Chromatin can apply either pushing or pulling forces onto the INM based on its compaction state. In addition, differences in the hydrostatic pressure between the nucleoplasm and the perinuclear space act on the INM, and the pressure difference between the perinuclear space and the cytoplasm acts on the ONM. Note, that some of these forces could act in opposite directions to those depicted here, depending on the direction of the external forces on the nucleus.

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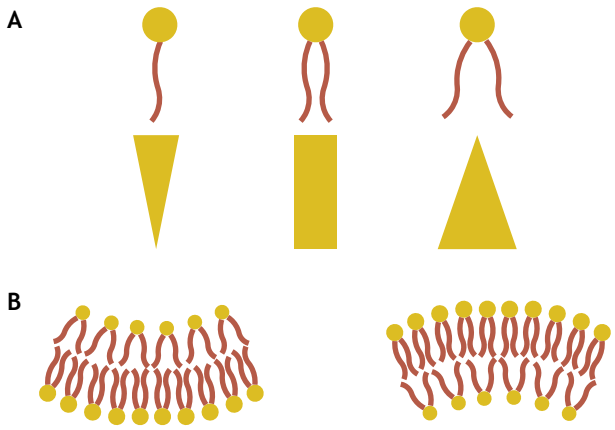

Fig. 3. Lipid composition impacts bilayer bending. (A) A lipid molecule can have three types of geometries – inverted cone, cylindrical or cone. (B) Depending on which leaflet is enriched in the cone-shaped lipid in a bilayer, the bilayer will bend into different directions.

red blood cells (Canham, 1970; Deuling and Helfrich, 1976; Jenkins, 1977), the ER (Guven et al., 2014; Terasaki et al., 2013) and mitochondria (Ghochani et al., 2010; Irajizad et al., 2019; Manor et al., 2015).

Impact of membrane mechanics on nuclear shape and nuclear membrane structure

Nuclear geometry in fission yeast

Nuclear membranes in yeast possess the same topology with a fused double membrane architecture as in mammalian cells. Unlike in mammalian nuclei, yeast membranes are not supported by a nuclear lamina as they do not express lamin proteins. Thus, these membranes lack the shear reinforcement that the lamina provides, and a model for nuclear mechanics needs to account primarily for nuclear membrane mechanics. Lim et al. modeled the yeast nucleus as an idealized single bilayer vesicle obeying the Helfrich–Canham model (Lim et al., 2007), and a similar vesicle model for multiple bilayers fused at pores has been investigated in (Noguchi, 2016). Lim et al. used their model to calculate nuclear shape transformations caused by microtubule elongation during closed mitosis in fission yeast (*Schizosaccharomyces pombe*) (Lim et al., 2007). The model accounted for forces stemming from microtubule growth, and exchange of lipids between the nuclear membrane and a lipid reservoir; lipid exchange allowed for large increases in nuclear surface area. This study suggested that the tension in the membrane and the pressure across the membrane due to the constrained internal volume may regulate the geometry of the interphase nucleus. Overall, this study highlighted the importance of considering lipid bilayer mechanics in understanding nuclear shaping in fission yeast (Lim et al., 2007).

Lipid bilayer mechanics is likely to also be important in nuclear fusion during yeast zygote formation. Experimental studies have revealed that the fusion of nuclei proceeds via a multistep process (Melloy et al., 2007; Tartakoff and Jaiswal, 2009), which involves fusion of the outer nuclear membranes, followed by fusion of inner membranes. Lipid bilayer fusion has been investigated in flat bilayers and spherical vesicles in several *in vitro* and modeling studies (reviewed in Chernomordik and Kozlov, 2008). The bilayer fusion process is controlled by membrane mechanics and chemical composition; in-plane tension and enrichment of cone-shaped lipids may catalyze bilayer fusion (Chernomordik et al., 1997; Marrink and Mark, 2003; Shillcock and Lipowsky, 2005).

Nuclear bilayer separation

As mentioned above, a distinct aspect of nuclear envelope structure is that the INM and the ONM are maintained at a relatively uniform distance of ∼35–50 nm. There are at least two interesting questions with respect to this separation: (1) what sets the separation length, and (2) how a spatially uniform spacing is maintained against the thermal and cytoskeletal forces that continuously act on the inner and outer membranes. Insight into these questions has been developed through a combination of experiments and computational calculations.

The separation length between nuclear membranes may be determined at least in part by the LINC complex (Rothballer et al., 2013). Depletion of SUN proteins is associated with a significant increase in the bilayer separation to nearly 100 nm in HeLa cells (Crisp et al., 2006). These findings are consistent with a model in which LINC complexes balance part of the cytoskeletal tensile force exerted on the ONM and also act as a spacer that sets the separation length scale. In the absence of the LINC complex, membranes will separate from each other when sufficiently large tensile stresses act on the ONM. Such stresses may not be homogeneously exerted on the nuclear surface, but might preferentially act at nuclear poles. For example, depletion of SUN proteins causes an increase in nuclear membrane separation preferentially at the anterior and posterior ends of muscle cell nuclei (Cain et al., 2014). Consistent with this view, an elastic shell model of the nuclear envelope predicted that the strain profile in the nucleus can be inhomogeneous (Cao et al., 2016), such that the leading end of the nucleus in a migrating cell will experience larger tensile stress.

However, depletion of SUN proteins does not always cause a change in nuclear bilayer separation; for example, there is no such effect in *Caenorhabditis elegans* embryos (Cain et al., 2014). This suggests that in the absence of the LINC complex, the cytoskeletal tensile stresses may be balanced by the nuclear membranes themselves, which might come under increased tension. In cells where cytoskeletal forces on nuclei are relatively low, it is possible that the membrane tension is large enough to resist external tensile stresses, and thus prevents the expansion of the membrane spacing even in the absence of the LINC complex.

In fission yeast, LINC complexes are restricted to the spindle body interface and are not uniformly distributed. However, the distances between nuclear membranes in fission yeast and in mammalian nuclei are similar. An additional complexity is that the tension in the nuclear membranes may not be spatially homogeneous; indeed, such inhomogeneity has been demonstrated in plasma membranes (Shi et al., 2018). In the context of nuclear membranes, the forces from various proteins (as shown in Fig. 2B) may similarly cause spatial variations in tension in the INM and the ONM. In any case, the mechanical state of the nuclear membrane itself is likely to be an important factor in regulating envelope spacing in yeast nuclei.

Consistent with the notion that nuclear membrane tension may have an important role in nuclear membrane structure, a computational approach that minimized bending and stretching energies using the Helfrich–Canham model (Torbati et al., 2016) predicted a decrease in the nuclear membrane separation with an increase in membrane tension. When the membrane was assumed to be under compression (negative tension), the bilayer separation increased significantly, eventually undergoing a bucking instability at a critical compressive stress. The study also provided an estimate of ∼0.1 mN/m in-plane tension in the nuclear membranes that yields the experimentally observed ∼45 nm separation. This tension is an order of magnitude higher than the typical plasma membrane

tension of ∼0.01 mN/m (Gauthier et al., 2012), suggesting that there are potentially fundamental differences between the mechanical behavior of nuclear membranes and that of the plasma membrane. However, the nuclear membrane does share some similarities with the plasma membrane at cell–cell contacts, where two plasma membranes from neighboring cells are in close proximity and bridged by E-cadherin linkages (Leckband and de Rooij, 2014). Similar to the nuclear lamina that underlies the INM, the plasma membrane also features a reinforcing underlying structure, the F-actin cortex. Furthermore, like the plasma membrane in cell–cell adhesions, the nuclear membranes may experience tensile forces from either side of the envelope (Mazumder et al., 2008).

A complicating factor in modeling the nuclear membrane is that the ONM is connected to the ER, and the INM is connected to the NR. The ER and NR may serve as reservoirs that exchange lipids through viscous flow with the ONM and the INM; such exchange of lipids can regulate membrane tension (Raucher and Sheetz, 1999). Indeed, flow between the nuclear membranes and the ER has been suggested as an explanation for the doubling of nuclear envelope area during the cell cycle in fission yeast and the maintenance of membrane tension (Lim et al., 2007). However, there is no direct experimental evidence of force-dependent lipid flow between the ONM and the ER so far.

Nuclear bilayer separation may also be affected by the composition of lipids in the nuclear membranes. Diacylglycerol (DAG) lipids are preferentially present in the INM of the nuclear envelope in *S. cerevisiae* (Romanauska and Köhler, 2018). DAG is a cone-shaped lipid and thus may generate a preferred curvature at the pore sites, thereby affecting both the pore geometry and the bilayer separation. Indeed, assuming preferred curvature near the nuclear pore and zero tension in the membrane, the Helfrich-Canham model predicts that the experimentally observed nuclear pore geometry is the energetically most optimal solution (Agrawal and Steigmann, 2009).

The mechanics of nuclear pore formation and pore spacing

An interesting aspect of nuclear membrane structure is the relatively regular spacing between adjacent nuclear pores. This spacing tends to be in the range of 250 to 500 nm across different cell types (Belgareh and Doye, 1997; D'Angelo et al., 2006; Dultz and Ellenberg, 2010). Because the nuclear membranes are ∼45 nm apart, formation of new pores in an intact nucleus during interphase can occur only through the bending of one membrane toward the other or bending of both membranes toward one another (De Magistris and Antonin, 2018). Indeed, super-resolution microscopy has shown that the INM bends toward the ONM, eventually leading to fusion (Otsuka et al., 2016). Bending of the INM by nucleoporin POM121 has been suggested to promote nuclear membrane fusion (Fichtman et al., 2010; Funakoshi et al., 2011), while there is another proposal that the ONM may bend toward the INM through the action of reticulon proteins (Talamas and Hetzer, 2011). From a mechanical standpoint, it is intriguing that the INM bends preferentially toward the ONM, because the INM is linked to the nuclear lamina through the binding of INM proteins such as emerin to the lamins (Ostlund et al., 2009; Vaughan et al., 2001). Therefore, the INM-lamina composite is expected to be stiffer and to resist bending more than the ONM.

One explanation for the observed membrane bending that leads to pore formation is that this bending may be a type of buckling instability that is induced owing to a build-up of in-plane compressive stresses in the membranes. Computational modeling using the Helfrich-Canham model suggests that the buckling response of the nuclear membrane is strongly dependent on the two-dimensional membrane area that lies in between adjacent nuclear pores (Torbati et al., 2016). The nuclear membrane is predicted to be highly stable against buckling if the membrane length between pores is less than 250 nm, while it is predicted to be highly unstable at lengths above 500 nm (Torbati et al., 2016). Thus, buckling is predicted to occur for membrane patches that are in the range of 250 to 500 nm diameters, which is consistent with experimental measurements of nuclear pore spacing (Belgareh and Doye, 1997; D'Angelo et al., 2006; Dultz and Ellenberg, 2010). Thus, the mechanical properties of the nuclear membranes, which govern their buckling behavior, may at least in part contribute to the emergence of the relatively uniform nuclear pore spacing observed in interphase cells.

The source of in-plane compressive stresses that may cause nuclear membrane buckling is not clear. One possibility is that addition of lipids to the membrane from the ER or arising from the fusion of lipid vesicles (Hetzer, 2010) with the nuclear membranes could cause compressive stresses. Supporting the latter possibility, *in vitro* studies have shown that vesicle fusion with membranes can generate a compression in membranes (Solon et al., 2006). Another possibility is that compressive forces could be generated by locally populating the membranes with proteins (Stachowiak et al., 2012). It is conceivable that proteins, such as POM121 or reticulons, which have been implicated in pore formation (Funakoshi et al., 2011), may directly generate compressive stresses in the membrane and bend the INM. Elucidating the contribution of these different sources to the bending process remains an important challenge.

Finally, the lipid composition of the INM may be a factor in its buckling behavior under compressive stresses. As mentioned before, the INM is enriched in DAG lipids, which have a natural propensity to bend the INM. The leaflet of the INM with a higher concentration of these lipids will determine whether the INM will bend toward the ONM or away from it. While the lipids favor bending the INM, such bending will likely be resisted by the lamina and thus create a confined environment for the INM. These opposing effects could generate a compressive stress in the INM that can eventually buckle it.

Extreme bending of the nuclear envelope

An example of extreme bending of mammalian nuclear membranes, where the mechanical properties of the membranes may be important, is the NR, which consists of invaginations of the nuclear membrane into the nucleoplasm (Fig. 1). The INM can invaginate into the nucleoplasm without any ONM bending, or both the INM and ONM can bend inward (Malhas et al., 2011; Malhas and Vaux, 2014). NR involving both INM and ONM is associated with the nuclear lamina, which consists of A- and B-type lamins, and forms around cytoskeletal structures in the cytoplasm, such as F-actin and cytoplasmic intermediate filaments (Jorgens et al., 2017). Whether NR is a consequence of in-plane membrane compressive stresses that arise from lipid recruitment or whether it forms owing to the mechanical stresses generated by the cytoskeleton is not entirely clear. It is also unclear why in some NR, only the INM invaginates, but not the ONM. Calculations of the energetics of lipid membrane bending in these structures, and dynamic imaging of the development of different types of NR may be useful in shedding further light on the mechanism underlying these structures.

Importance of nuclear membrane mechanics in the context of nuclear rupture

Single lipid membranes are only able to undergo 2–5% areal stretch, beyond which they rupture (Rawicz et al., 2000). A recent

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development relevant in the context of nuclear membrane mechanics is that fused nuclear membranes have also been observed to undergo rupture in cells (Denais et al., 2016; Hatch and Hetzer, 2016; Raab et al., 2016; Zhang et al., 2019). Membrane rupture is detrimental to cells as it exposes nuclear contents to the cytoplasm and *vice versa*, causing DNA damage, which has negative consequences for cell function (Denais et al., 2016; Irianto et al., 2017). How the nuclear membranes rupture is not fully understood, but it is clear that the rupture is caused by mechanical stresses.

When membranes rupture, what determines the size of the resulting hole? When a single membrane ruptures, the energy of the resulting hole is determined by two parameters, the membrane tension and the line tension. Line tension arises from the extreme bending of lipids at the exposed edge of the hole, which entails an energetic cost (Fig. 4). Membrane tension tends to pull the membrane out to expand the hole, while the line tension opposes pore expansion. The ratio between the line tension and membrane tension then determines the equilibrium geometry of the hole (Gonzalez-Rodriguez et al., 2012).

Based on typical values of membrane tension and line tension, single-lipid membrane holes tend to be a few nanometers in size (Akimov et al., 2017a,b). Once formed, the hole can expand, relocating lipids from the hole to the rest of the membrane surface; this can lower the areal stretch and hence, reduce the membrane tension. Therefore, large stable holes of up to a micron in diameter can potentially be obtained if continual lipid relocation decreases the areal stretch after rupture (Gonzalez-Rodriguez et al., 2012).

Nuclear membrane structure and associated proteins may protect against rupture

The rupture tension of a typical lipid membrane is estimated to be ∼8–10 mN/m (Rawicz et al., 2000). Build-up of tension in the nuclear membrane to this lytic tension will thus cause a rupture. Because the membranes may be mechanically reinforced against rupture by the LINC complexes, the nuclear pore complexes, and the underlying nuclear lamina and chromatin, it is not straightforward to predict what magnitude of stresses applied to either the ONM or the INM will cause the in-plane nuclear membrane tension to build up to the lytic tension. Understanding this will require parsing of the differential transmission of stresses by these different components. This may be a non-trivial task because perturbing one component of the nuclear envelope or chromatin will likely impact other components. For example, depletion of nuclear lamins to manipulate the nuclear lamina may disengage the inner nuclear membrane from the lamina, resulting in more pliable nuclear membranes. Nuclear lamin depletion may also impact the nuclear membrane mechanics indirectly by altering the distribution of LINC complexes and/or promoting the mobility of INM proteins, such as emerin, that are otherwise bound to the lamina. Such alterations may reduce the membrane viscosity, enhance lipid flow between the two nuclear membranes and/or alter the tension in the two membranes.

Furthermore, altering the chromatin state may impact on nuclear envelope mechanics. For example, decompaction of the nucleus due to an increase in euchromatin or decrease in heterochromatin have been shown to initiate nuclear membrane bleb formation (Stephens et al., 2017). Outward chromatin herniation has been suggested to precede and potentially cause nuclear envelope rupture (Hatch and Hetzer, 2016). Studies with *S. pombe*, which lack lamins, have shown that the linkage between chromatin and INM that is mediated by INM proteins Heh1, Heh2 and Man1 allows the nucleus to resist external mechanical stresses (Schreiner et al., 2015). Decondensed chromatin may exert an entropic outward-pushing force on the membranes, while condensing chromatin may generate an inward-pulling force on them; a balance between these opposing forces has been observed, at least in isolated nuclei (Mazumder et al., 2008). Thus, chromatin can alter nuclear envelope mechanics both by pushing on it or by pulling on it through INM linkages, and thereby impact the rupture process.

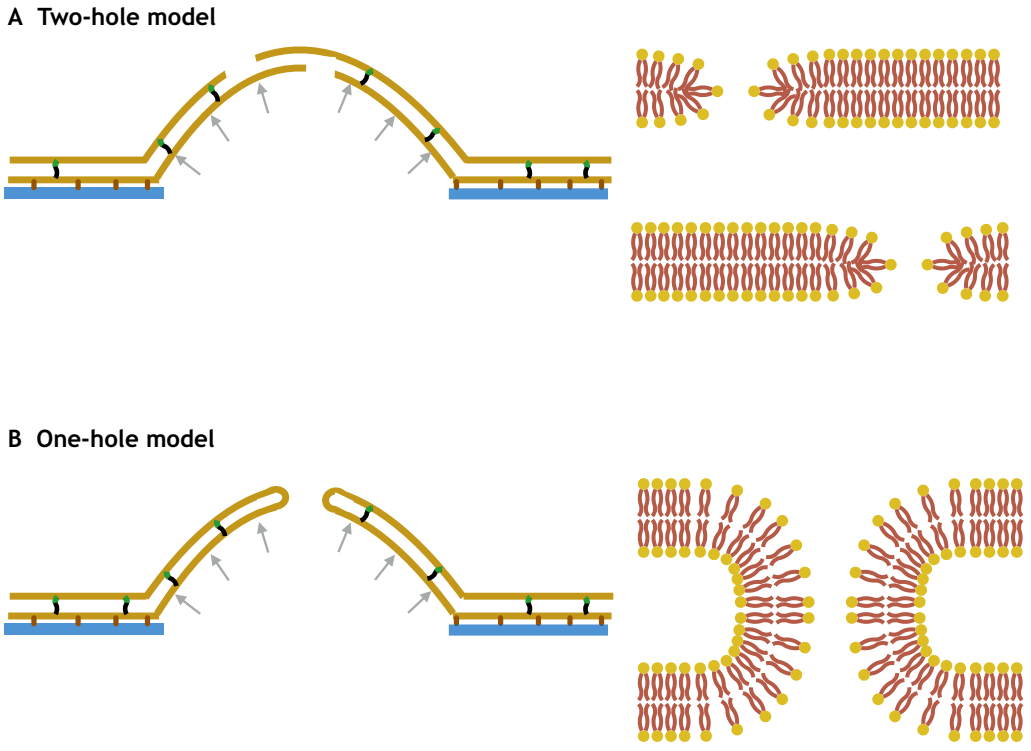

Fig. 4. Possible modes of hole formation mechanisms in ruptured nuclei. (A) The ONM and INM may each have a hole after rupture (two-hole model). At the edge of these holes, lipids will undergo extreme bending (over a distance of 4–5 nm) to avoid exposure of hydrophobic tails to the aqueous medium (shown on the right). Such deformation costs energy, which manifests in the form of a line tension and opposes further expansion of the hole. (B) Alternatively, in the one-hole model, the ONM and INM can fuse with each other after rupture to form a donut-shaped single hole (shown on the right). A donut-shaped hole has no edge and hence has no line tension energy; therefore it allows for larger hole sizes of 40–50 nm, consistent with experimental observations. The gray arrows indicate the net outward pressure acting on the membrane, and the thick blue line indicates the nuclear lamina.

Recent biophysical studies have begun to provide insights into the mechanical state of the nuclear membranes upon rupture. A model of the nuclear envelope as a viscoelastic shell and chromatin as a semiflexible polymer predicted an initial exponential growth of ruptured membrane holes followed by chromatin herniation; after this the hole size was predicted to decrease linearly with time (Deviri et al., 2017). The reduction in hole diameter was predicted to occur because of the release of internal pressure due to leakage of nuclear contents across the nuclear envelope, which reduces the in-plane tension in the membranes.

Super-resolution imaging studies have suggested that the size of holes in ruptured nuclear membranes is of the order of 100 nm (Denais et al., 2016). This experimental evidence is supported by estimates of the hole size from measurements of the kinetics of fluorescent probe decay from the nucleus upon rupture (Zhang et al., 2019). These sizes are much larger than hole sizes for single bilayer membranes, which tend to be of the order of a few nanometers. One proposed explanation for the large and stable size of nuclear membrane holes is that holes in the ONM and INM may be fused together to form donut-shaped holes (Zhang et al., 2019) (Fig. 4). A donut-shaped hole has no edge and hence has no line tension energy. The hole energy comprises the bending energy of the membrane owing to the donut shape at the hole site and the stretching energy. Equilibrium calculations of donut-shaped holes in nuclear membranes predict hole sizes post-rupture of the order of 100 nm, and can also explain the reported dependence of hole size on applied stress (Zhang et al., 2019).

Upon nuclear rupture, the nuclear membranes can undergo repair, such that the newly formed hole is completely sealed (Denais et al., 2016; Hatch and Hetzer, 2016; Vargas et al., 2012; Zhang et al., 2019). The endosomal sorting complexes required for transport III (ESCRT III) machinery may contribute to hole repair (Denais et al., 2016; Raab et al., 2016). The molecular pathway by which ESCRT-III proteins or related proteins may repair nuclear membranes is not entirely clear, but a mechanism has been proposed for how these proteins might deform single lipid bilayers *in vitro* (Chiaruttini et al., 2015). ESCRT III polymerizes as a spiral on the membrane surface and becomes compressed. When the compression is released, the spiral can undergo out-of-surface deformation, thereby bending the underlying membrane. In the context of nuclear membranes, the cost to bend the membranes may be significantly larger because of their fused geometry. Further studies are needed to understand the membrane deformation pathways – that is the evolution of the membrane shapes, and the associated energetics of deformation – which ESCRT III or other as yet unknown proteins harness to repair nuclear membrane holes.

Conclusions and perspectives

Much insight has been gained into nuclear envelope structure and the composition of envelope proteins, but relatively less is known about the mechanical properties of the nuclear membranes. It is clear that, given their geometry and the fact that there are several reinforcing proteins that span the envelope, and the nuclear lamina that underlies the INM, the mechanical rigidity of the envelope will be quite different compared to that of single bilayer membranes and also the plasma membrane.

The nucleus is constantly exposed to cellular mechanical forces, which are resisted by various components in the nucleus, and also the nuclear membranes that comprise the nuclear envelope. Therefore, it is critical to understand the mechanical properties of the nuclear envelope and the effect of mechanical forces on its structure and dynamics, as well as how envelope proteins protect it against rupture. We anticipate that the field will benefit from measurements of the bending rigidity of nuclear membranes under calibrated mechanical forces and of in-plane membrane stresses, as well as computational calculations of membrane properties and morphological transformations. Measurements of nuclear membrane mechanics could possibly be performed using methods that have been used previously for quantifying plasma membrane mechanics (Gauthier et al., 2012). The field will also benefit from recently developed *in vitro* model systems involving reconstituted artificial nuclear membranes that contain the LINC complex (Majumder et al., 2019).

Defects in the nuclear envelope have been linked to a range of human pathologies, including tumorigenesis and laminopathies (reviewed in Malhas and Vaux, 2014; Ungricht and Kutay, 2017), and it is possible that such defects at least in part may be related to altered nuclear membrane mechanics. A thorough characterization of nuclear membrane mechanics may therefore be essential to yield a complete understanding of these pathologies.

Competing interests

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