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A model for coordinating nuclear mechanics and membrane remodeling to support nuclear integrity

Megan C. King^{*,&} and C. Patrick Lusk^{*,&}

^{*}Department of Cell Biology, Yale School of Medicine, New Haven, CT

Abstract

A polymer network of intranuclear lamin filaments underlies the nuclear envelope and provides mechanical stability to the nucleus in metazoans. Recent work demonstrates that the expression of A-type lamins scales positively with the stiffness of the cellular environment, thereby coupling nuclear and extracellular mechanics. Using the spectrin-actin network at the erythrocyte plasma membrane as a model, we contemplate how the relative stiffness of the nuclear scaffold impinges on the growing number of interphase-specific nuclear envelope remodeling events, including recently discovered, nuclear envelope-specialized quality control mechanisms. We suggest that a stiffer lamina impedes these remodeling events, necessitating local lamina remodeling and/or concomitant scaling of the efficacy of membrane-remodeling machineries that act at the nuclear envelope.

Introduction

The defining organelle of eukaryotes is the nucleus, which physically segregates the genome from the cytoplasm. This compartmentalization is achieved by the nuclear envelope (NE), a membrane system continuous with the endoplasmic reticulum (ER). As such, the NE is formed from a single lipid bilayer that is biochemically and functionally segregated into three distinct membrane subdomains: the inner nuclear membrane (INM), the outer nuclear membrane (ONM) and the pore membrane, which connects the INM and ONM and houses the massive nuclear pore complexes (NPCs)(Fig. 1). While it is intuitive (and well understood) that the NE controls the segregation of nuclear and cytosolic contents, it is also increasingly clear that the NE serves as a landmark for the coordination of processes regulating the genome including transcription and DNA repair [1-4]. Moreover, because the nucleus is typically the largest and most stiff organelle, it is uniquely susceptible to mechanical stresses imposed by both external and internal forces [5]. These forces are buffered by connections between the INM and an underlying nuclear scaffold built from the intermediate filament lamins and connections to chromatin through INM proteins [6,7](Fig. 1).

[&]Corresponding authors: patrick.lusk@yale.edu, megan.king@yale.edu.

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The emphasis in the field on the critical role for the A- and B-type lamin polymer networks in providing mechanical stiffness to the nucleus has led to the perspective of the NE as a fixed, stable structure, particularly in post-mitotic cells. Consistent with this idea, the lamina effectively immobilizes NPCs [8] and many INM proteins within the NE [9-12], supporting the concept that the nuclear lamina is a highly interconnected, multivalent network of protein-protein and protein-membrane interactions. Remarkably, this network is further mechanically integrated into the cell, perhaps even extending out to cell-cell junctional complexes through direct cytoskeletal linkages to LINC complexes that span the NE [13] (Fig. 1). Together these architectural principles can lead one to imagine that this robust network maintains a rigid NE domain.

This static view of the interphase NE is being challenged by new evidence that the NE is subject to dynamic membrane remodeling [14,15]. New work has introduced established membrane remodeling machines like the Endosomal Sorting Complexes Required for Transport (ESCRTs) and the ER/NE-lumenal AAA+ ATPase torsin to well known NE remodeling events like those that facilitate *de novo* NPC assembly [16,17], but also to the 'alternative' transport of mega-ribonucleoprotein particles (RNPs) through a virus-like budding process through the NE [18]. Further, dramatic NE blebbing events have been observed during an autophagy-mediated process that is capable of specifically removing and degrading lamin B1 from the INM under conditions that mimic cellular transformation [19]. This work harkens back to the discovery of piece-meal microautophagy of the nucleus (PMN) nearly 15 years ago [20,21], as well as the more recently uncovered nucleophagy pathway [22]. These new insights raise critical questions about how to reconcile the view of the NE (and particularly the INM) as a mechanically rigid, membrane-anchored scaffold with evidence that the NE membranes are subject to numerous remodeling events. Moreover, if these two systems are fundamentally in conflict, how might they be co-regulated to provide the nucleus with both the dynamics and mechanical strength it requires to carry out its many functions?

Conceptual parallels between the lamina and the spectrin-based membrane skeleton

Classical studies of erythrocyte membranes and particularly the interplay between the spectrin protein network, membrane biophysics and endocytosis provides a valuable framework in which to consider how the properties of the nuclear lamina may impact NE remodeling (Fig. 2). In erythrocytes, a membrane-anchored 2D polygonal protein cytoskeleton composed largely of spectrin is integrated with the actin network to provide mechanical stability to the erythrocyte membrane [23]; a similar (but architecturally unique) network provides mechanical support to other cellular membranes, for example in axons [24]. Seminal experiments using erythrocyte ghosts revealed that this spectrin network inhibits endocytosis; this inhibition can be locally relieved by spectrin disassembly [25]. This concept likely applies more broadly as recent work in epithelial cells supports the existence of membrane-associated spectrin "microdomains" that inhibit clathrin-mediated endocytosis [26], while the cortical actin network has long been suggested to impede exocytosis in a variety of cell types [27,28]. This leads to a model in which membrane-associated protein networks can inhibit membrane deformation in stark contrast to the numerous protein coats that support membrane remodeling by driving (or stabilizing)

membrane curvature [29]. Interestingly, phosphoregulation of the spectrin network regulates its assembly state ([30]; Fig. 2A). Thus, while the extent of the spectrin network can define a “set point” for the accessibility and/or susceptibility to membrane remodeling, this property can be rapidly altered either locally or globally to become permissive to membrane remodeling (Fig. 2A).

Much like the actin-spectrin cytoskeletal network at the plasma membrane, the nuclear lamina is composed of distinct of A- and B-type lamin polymer assemblies [31] that are tethered both directly and indirectly (through lipid modifications or interactions with integral membrane proteins, respectively) to the INM [7](Fig. 1 and 2B). It remains largely unasked how the lamina impacts the various membrane remodeling events that have recently been revealed to be essential for maintaining NE composition, organization, function, dynamics and quality control (QC) [15]. It is clear that nuclear mechanics scale with the mechanical environment in which the cell resides (so-called “mechanoreciprocity” [32,33]), thereby establishing distinct mechanical “set points” driven by global differences in lamin A expression level and/or turnover [33-35], the degree of lamin A phosphorylation on serine 22 ([35]; the classic “mitotic”, pro-disassembly modification [36,37]), the lamin assembly state [38] and the extent of lamina-associated heterochromatin [39,40]. To accommodate different mechanical set points, local remodeling of the nuclear lamina could be achieved by driving lamin A Ser22 phosphorylation (and potentially the phosphoregulation of other lamina components), in direct analogy to the spectrin phosphorylation that makes the erythrocyte membrane permissive to endocytosis (Fig. 2).

Consistent with a model of local lamina remodeling, the local disruption of the nuclear lamina by phosphorylation has been suggested to be an early event in *de novo* NPC assembly [41], although (to our knowledge) this concept has not been formally tested (Fig. 3A). Further, the egress of herpesvirus from the nucleus is enabled by viral and host-encoded kinases like protein kinase C (PKC) [42] that phosphorylate the lamins (Fig. 3B). More recently, lamin-phosphorylation by PKC has been implicated in an analogous egress mechanism observed in *Drosophila* neuromuscular junctions, where so-called “mega” RNPs too big to fit through NPCs cross the NE in a vesicular intermediate that transits the NE lumen/perinuclear space (PNS) [43] (Fig. 3B). Together these data support the notion that local lamina disassembly may be a fundamental requirement for efficient NE remodeling. An interesting question to consider is whether the suppression of INM evagination occurs simply by steric exclusion of the membrane remodeling machinery or whether a “templating” of the membrane sheet to the 2D proteinaceous scaffold may perhaps energetically oppose the induction of membrane curvature (and indeed, even thermal membrane fluctuations).

Quality control at the NE

Another broad class of NE remodeling events that likely requires coordination with local changes in the nuclear lamina includes various NE-specific QC pathways that have been the topic of several recent studies [15,16,19,22,44,45]. For example, expression of an activated form of the oncogenic H-Ras GTPase (HRasV12) leads to the specific degradation of lamin B1 [19]. This pathway is thought to proceed through the engulfment of lamin B1 containing

NE blebs by the cytosolic autophagy machinery [19] (Fig. 3C). As lamin B1 is specifically recognized by binding to the activated form of LC3 (a key component of the autophagy machinery that is covalently coupled to phosphatidylethanolamine; [46]) the lamin B1-LC3 interaction could occur at the INM. While one can conceptualize the internalization of LC3-lamin B1 into an INM evagination, it becomes more challenging to envisage how such an evagination could lead to the pinching off of a double-membrane vesicle containing portions of both the INM and ONM into the cytosol, particularly since LC3 would be hidden from downstream autophagy components necessary for fusion with the lysosome (Fig. 3C). Nonetheless, the direct visualization of the formation and pinching off of nuclear blebs containing lamin-B1 make a compelling case for the existence of this autophagy pathway; a critical next step is to identify the molecular machinery that is capable of deforming, budding, and resolving the two lipid bilayers of the NE.

The lamin B1-QC pathway likely involves additional molecular players outside of the canonical autophagy pathway, as it is not stimulated by traditional autophagy inputs like starvation or down-regulation of the TOR pathway [46]. Interestingly, however, in yeast there is evidence that autophagic mechanisms operate during nutrient deprivation to drive the turnover of nuclear components. PMN was discovered ~15 years ago [20] (Fig. 3D), and requires specific NE-vacuole/lysosome junctional proteins (this work also describes one of the first biochemical characterization of an organelle contact site) that promote the engulfment of portions of the nucleus and NE by the vacuole (Fig. 3D). While PMN requires known autophagy factors [47], an additional nucleophagy pathway that may be mechanistically distinct has recently been described [22] (Fig. 3E). This work identified two novel autophagy receptors, Atg39 and Atg40; the discrete segregation of these receptors between the NE (Atg39) and the peripheral/cortical ER (Atg40) is reminiscent of the segregation of ERAD components [44,45] suggesting that they too define distinct arms of a more general “ER-phagy” mechanism. It is likely that the recently identified FAM134B [48] is the mammalian equivalent of Atg40, although a clear orthologue of Atg39 remains to be identified. Nonetheless, the appearance of lysosomes that abut the NE containing nuclear contents is suggestive that an analogous nucleophagy pathway exists in multicellular eukaryotes as well [49].

Membrane remodeling at the NE: specific adaptors for established machineries?

A common thread between the nuclear viral/mega-RNP egress, the lamin B1-LC3 and the nucleophagy pathways is the removal of proteins, nucleic acids and membrane from the inside of the nucleus; topologically, these processes require membrane remodeling machineries to either evaginate the INM and/or to seal the NE after it is engulfed (Fig. 3). In the latter case, a clear candidate is the ESCRT machinery, which has recently been shown to seal NE holes in order to complete NE reformation at the end of mitosis [50,51]. Interestingly, the ESCRT machinery has also been implicated in a QC mechanism that ensures the proper formation of NPCs through the clearance of defective NPC assembly intermediates at the NE [16]. Indeed, the physical interactions between the integral INM proteins Heh1 and Heh2 with the ESCRT machinery [16], coupled to its inherent capacity to drive membrane deformation and/or membrane scission [52-56], suggests it could be a key component of several pathways that require INM evaginations, including the *de novo*

assembly of NPCs into an intact NE [57](Fig. 3A). Further studies will be needed to assess the potential role for the ESCRT machinery in these mechanisms.

Interestingly, while the machinery that drives membrane remodeling and fusion has not been definitely established during *de novo* NPC assembly (although growing evidence supports a local confluence of proteins capable of recognizing and stimulating membrane curvature [58-69]; Fig 3A), recent work in *C. elegans* suggests that the AAA+ATPase torsin orthologue (OOC-5) might contribute to these events, as depletion of OOC-5 leads to the accumulation of clustered NPCs at the NE, a classic NPC misassembly phenotype [17]. Interestingly, torsin alone does not function as an efficient ATPase; its active site is formed through binding to either the integral INM protein LAP1 or the ER membrane protein LULL1, making torsin a unique lumenal-targeted member of the AAA+ ATPase family that might have distinct substrates in different NE/ER subcompartments [70,71]. Significant effort is being directed towards identifying these substrates [72]; these will very likely include NE components, as depletion or mutation of torsin in some cell types leads to the accumulation of INM evaginations [17,18,73]. These data suggest that torsin might be involved with resolving INM-buds to form intraluminal vesicles in a mechanism that is required for mega-RNP egress [18], or perhaps even the removal of protein aggregates from the nuclear interior [74] (Fig. 3B). Resolving the necks of the INM evaginations would require a membrane fission process that is topologically-related to those carried out by the ESCRT machinery (Fig. 3B). Whether there is a functional interplay between the ESCRTs and torsins will be a challenge for future work. Instead, it is also possible that ESCRTs and torsins act in parallel pathways, as yeasts lack torsin orthologues yet are nonetheless capable of undergoing the same membrane remodeling events [57].

Conclusion and Outlook

For many years, the field has sought to identify the factors required for NE remodeling, particularly in the field of NPC biogenesis, where the identification of a single membrane bending/fusion machinery remains elusive [75]. The recent explosion of studies demonstrating NE-specific roles for common cellular machines that act in diverse cellular compartments, like components of the ESCRT, torsin and autophagy pathways, likely explains one reason why such factors have been challenging to identify. In this context, a key question going forward will be the identity of the NE-specific adaptor molecules that regulate the contextual and kinetic timing of recruitment of these machineries to execute the membrane remodeling events necessary for NE homeostasis. Importantly, we would argue that these adaptors must also be coordinated with the mechanical set point of the nuclear lamina, either globally or locally (Fig. 4). In the global model, the “power” (e.g. protein levels, recruitment, or activation) of the membrane remodeling machinery would be tuned to the global mechanical “set point” of the nucleus to support necessary NE remodeling (Fig. 4). Alternatively, a greater need for factors that promote local lamina disassembly (e.g. kinase activity) would be required to support membrane remodeling in the context of stiff nuclei. Either model suggests that the hallmark changes in nuclear shape and structure that occur in disease could result from a misregulation of this delicate balance [14,76].

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Figure 1. Schematic of the nuclear envelope

Key components of the NE are shown including the inner and outer nuclear membrane (INM and ONM), which enclose the perinuclear space (PNS). Nuclear pore complexes (NPCs) span the NE and are formed from several unique subcomplexes of nucleoporins that form scaffold complexes (orange, purple and violet) and the nuclear basket (maroon and green). Nucleoporins rich in Phe-Gly amino acid residues (blue squiggles) line the central transport channel. LINC complexes containing “SUN” and “KASH” domains connect chromatin (orange/black fibres) to cytoskeletal elements. Other conserved INM proteins are shown. Torsin is a component of AAA+ ATPase complex formed by binding to the INM protein LAP1. A- and B-type lamins (light blue and light brown, respectively) form a polymer assembly that provides mechanical stability to the NE and binding sites for chromatin.

Figure 2. Conceptual and physical parallels between the spectrin and lamin membrane scaffolds

A. A network of α - and β -spectrin proteins (red and black lines) underlies the plasma membrane of many cells including erythrocytes. The local phosphorylation (yellow P) of the spectrins leads to their local disassembly, which facilitates membrane remodeling required for endocytosis. **B.** The nuclear lamina is composed of distinct networks of A- and B-type lamins (blue) that provide mechanical stability to the nuclear envelope by interacting (directly and indirectly) with the inner nuclear membrane (INM). As with the spectrin network, it is likely that phosphorylation of the lamina enables membrane remodeling of the INM. ONM and PNS are outer nuclear membrane and perinuclear space, respectively.

Figure 3. Processes requiring membrane remodeling at the nuclear envelope

A. Nuclear pore complex (NPC) biogenesis likely occurs through the initial recruitment of scaffold complexes to the inner nuclear membrane (INM) by either integral INM proteins (yellow) or nuclear basket components (maroon). There is evidence to support the involvement of LINC complexes [77] or other luminal bridging proteins [78] that could connect the INM to the outer nuclear membrane (ONM). This recruitment is prior to (or concomitant with) membrane remodeling that likely drives evaginations of the INM (and/or invaginations of the ONM). It remains unclear whether there is a single membrane-bending

machinery, like the ESCRTs or torsin-substrates, or whether it is a local concentration of curvature sensing/generating reticulon-like proteins (black W shapes) and nucleoporins with amphipathic helices (purple squiggles) that drive local membrane remodeling. Orange asterisks denote locations of membrane remodeling. **B.** Pathway of nuclear egress used by viruses and “mega” RNPs, and perhaps, defective intermediates in NPC assembly (all exemplified by red circle). Either torsin or ESCRT-III might contribute to membrane scission to form an intraluminal vesicle after local lamina disassembly by phosphorylation (yellow P). **C-E.** Examples of autophagy that occurs at the NE by direct engulfment of the NE and nuclear contents by the vacuole or phagophore (brown). Alternatively, nuclear blebs containing nuclear material pinch off the NE and are engulfed by a phagophore in the cytosol. The selective degradation of lamin B1 in the context of HRasV12 overexpression is mediated, in part, by a direct interaction with the activated lipid-bound form of the autophagy protein, LC3 (green). Likewise, nucleophagy occurs by binding of Atg39 to the LC3 equivalent in yeast. See legends at right.

Figure 4. Proposed balance between nuclear mechanics and nuclear envelope dynamics

Top panel shows a scale depicting the concept of a balance between nuclear mechanics and nuclear envelope dynamics that would be tuned to distinct tissue-specific mechanical “set points”. We suggest that the tuning could be at the level of the “power” of NE-remodeling machineries, which would need to be stronger in contexts of nuclei in stiff extracellular environments. Alternatively, access to (or susceptibility to) the membrane remodeling

machinery could be promoted by local remodeling of the lamina. Disruption of this balance might contribute to loss of NE integrity, a typical phenotype associated with laminopathies.

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