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Messages from the voices within: regulation of signaling by proteins of the nuclear lamina

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Abstract

The nuclear lamina (NL) is a protein scaffold lining the nuclear envelope that consists of nuclear lamins and associated transmembrane proteins. It helps to organize the nuclear envelope, chromosomes, and the cytoplasmic cytoskeleton. The NL also has an important role in regulation of signaling, as highlighted by the wide range of human diseases caused by mutations in the genes for NL proteins with associated signaling defects. This review will consider diverse mechanisms for signaling regulation by the NL that have been uncovered recently, including interaction with signaling effectors, modulation of actin assembly and compositional alteration of the NL. Cells with discrete NL mutations often show disruption of multiple signaling pathways, however, and for the most part the mechanistic basis for these complex phenotypes remains to be elucidated.

Introduction

The nuclear envelope (NE) forms the membrane boundary of the nucleus and thereby compartmentalizes the genome and its metabolism. It is a specialized ER subdomain containing an outer nuclear membrane (ONM) that is continuous with, and functionally similar to the peripheral ER, and an inner nuclear membrane (INM) with distinctive properties (Figure 1). The ONM and INM are joined at nuclear pore complexes (NPCs), ~100 MDa macromolecular assemblies that mediate molecular flux between the nucleus and the cytoplasm. In metazoans, the INM is lined by the nuclear lamina (NL), a protein meshwork consisting largely of nuclear lamins, type V intermediate filaments proteins [1,2]. The NL also contains numerous less abundant transmembrane proteins that directly or indirectly associate with lamins. Three major lamin subtypes are present in mammals. These are lamins B1 and B2, which are expressed in most cell types throughout development, and lamins A/C, products of alternative splicing that usually appear late during differentiation.

The NL has attracted widespread interest because of its expansive functional repertoire. It has been implicated in nuclear structure and mechanics, tethering of heterochromatin and the cytoplasmic cytoskeleton to the NE, and regulation of signaling and gene expression [2].

Consistent with this functional palette, mutations in the genes for lamins and associated transmembrane proteins have been found to cause a spectrum of human diseases (termed 'laminopathies') [3]. By far the largest number of laminopathy mutations occurs in the gene encoding lamins A/C. The most prevalent laminopathies are skeletal muscle dystrophies and cardiomyopathies. Less frequently the diseases can affect adipose tissue, peripheral nerve or lens [4], or target multiple tissue systems as exemplified by the premature aging-related disorder Hutchinson–Gilford progeria syndrome (HGPS) [3].

Diverse signaling pathways that are fundamental to cell behavior and differentiation are perturbed in animal and cultured cells models of laminopathies (Table 1) [5]. Mounting evidence from pharmacological and genetic analysis of mouse models indicates that many of these signaling perturbations may contribute to disease pathogenesis [5,6,7^{••},8–10]. Understanding the molecular basis for these aberrations, and more generally, the role of NL proteins in regulating signal transduction, is a central challenge for the field. This review will consider major signaling pathways that have been linked to NL proteins, some mechanistic underpinnings that have been revealed, and others that remain to be elucidated.

The regulatory framework at the inner nuclear membrane

The functional identity of the INM is dictated by nuclear lamins and its distinctive set of transmembrane proteins (Figure 1). As members of the intermediate filament family, lamins contain an ~50 nm long α -helical rod domain formed by a dimeric coiled-coil, flanked by non-helical head and tail domains. The different lamin subtypes are assembled into structurally similar filaments that form a densely interwoven meshwork apposed to the INM [11,12]. Recent cryo-electron tomography revealed that lamin filaments in cultured cells are unexpectedly thin: they have an ~3.5 nm diameter, and most likely contain two dimers per filament cross-section [13^{••}]. By contrast, cytoplasmic intermediate filaments have a diameter of ~10 nm [14]. The thin architecture of lamin filaments in proliferating cells could promote flexibility and structural dynamics to accommodate NE growth and repair. However, lamin organization in terminally differentiated cells remains to be determined. Interestingly, the mutations in lamins A/C causing striated muscle disease are dispersed throughout the head, tail and rod domains, which all contribute to lamin self-assembly [14]. This suggests that the disease phenotypes may result from changes in system-wide properties of the NL, such as its composition and/or supramolecular organization.

Some relatively abundant NL-associated transmembrane proteins have been analyzed in detail (Figure 1) [2]. They include four mammalian polypeptides containing a LEM domain (LAP2 β , emerin, MAN1 and LEM2), an ~45 residue segment that binds to the chromatin protein Baf [15]. LEM proteins are suggested to redundantly support lamin A-dependent anchoring of chromatin to the NL [16^{••},17]. The lamin B receptor (LBR), an INM protein that associates with the heterochromatin protein HP1, provides a second major mechanism for chromatin attachment to the NE [16^{••}]. Interestingly, LBR also functions as a sterol C14 reductase essential in certain cells, and this activity is disrupted by human disease mutations [18[•]]. A transmembrane assembly consisting of SUN domain proteins of the INM and nesprins of the ONM, termed the LINC complex, connects actin filaments, microtubules and intermediate filaments to the nucleus [19,20]. The SUN domain proteins interact with lamins

as well as with emerin and Samp1/Tmem201, and likely occur in dynamic complexes. The different SUN domain proteins [21] and nesprin types [19,20] have distinctive functional properties (Figure 1, legend), but little is known about the pairing of different SUN/nesprin forms.

Aside from the proteins discussed above, proteomics has identified numerous relatively low abundance transmembrane proteins in NE fractions [22–24]. Conventional subcellular fractionation and immunolocalization approaches have shown that some of these, including Myorg/Net37 [25] and Plpp7/Net39 [26], are enriched at the NE. New superresolution microscopy approaches are emerging [27] to evaluate whether such transmembrane proteins are concentrated at the NE relative to the peripheral ER (*de facto* implying a distinctive nuclear function), or simply are ER residents that are not NE-excluded [28*].

An emerging consensus is that many transmembrane proteins localize to the INM in mammalian cells by lateral diffusion in the lipid bilayer from the peripheral ER to the INM (Figure 1), where they are retained by binding to lamins, chromatin or other INM proteins [29]. However, in certain cell types and functional states, some NL-associated transmembrane proteins can be partially relocalized to the peripheral ER [30,31,32**,33]. This can have important regulatory consequences, as considered below for emerin.

Coupling the cytoplasm and nucleus via the LINC complex

By directly connecting cytoskeletal systems to the NL, the LINC complex plays a vital role in the nuclear movement and positioning associated with cell motility and differentiation [19,20]. How the LINC complex is reorganized at the nuclear surface to support these processes is a major unanswered question. A recent breakthrough revealed that the INM protein LAP1 and the ER luminal protein TorsinA (Figure 1) are needed for LINC complex-dependent nuclear movement in fibroblasts [34**]. LAP1 is a potent activator of TorsinA, an AAA+ ATPase linked to the movement disorder torsion dystonia [35*,36,37**]. Whereas AAA+ ATPases typically unfold or disassemble proteins, the substrates of the LAP1/TorsinA complex are unknown. The recent study [34**] suggests that these may include the LINC complex or associated components. Other signaling-related functions have been suggested for TorsinA [36], including a role in regulating lipid metabolism [38*].

Another major function of the LINC complex involves transmission of mechanical signals from the cytoskeleton to the nucleus [39], including information on the rigidity of the extracellular matrix. Nuclear stiffness, which is dictated largely by the mechanical properties of the NL [40], is adjusted to conform to extracellular matrix rigidity [39]. Reciprocal flow of mechanical information from the nucleus to the cytoskeleton can occur as well [34**,39]. Mechanical signals have been shown to modify the structure of the NL, in part by changing the levels and assembly state of lamins A/C [41] and the interactions or posttranslational modifications of emerin [32**,42**]. Some of these changes can affect gene expression, as discussed below.

Signaling control by interaction of transcriptional regulators with NL proteins

The best-characterized mechanism for signaling control at the NL involves the direct interaction of core signaling factors or transcriptional activators with NL proteins. This typically restricts the activity of these pathways (Table 1). This mechanism first was revealed in studies of MAN1, which attenuates BMP/TGF- β signaling [43–46]. MAN1 directly binds R-Smads, effectors activated in the cytoplasm by BMP/TGF- β receptor-mediated phosphorylation. This binding sequesters Smads at the nuclear periphery and limits their binding to transcriptional coactivators [43–46]. MAN1 also might provide a scaffold for phosphatase-mediated inactivation of Smads [47]. Emerin has been shown to negatively regulate Wnt/ β -catenin signaling by a related mechanism [10,48,49]. Activation of Wnt signaling involves the stabilization of β -catenin in the cytoplasm, and this promotes its nuclear accumulation and binding to gene promoters. A short region in emerin directly binds to β -catenin and is responsible for its depletion from the nucleus and Wnt inhibition [48]. Whether the emerin interaction potentiates nuclear export of β -catenin, or simply promotes its intranuclear turnover, is unknown. Recently, emerin also has been suggested to restrict Notch signaling by binding to the Notch intracellular domain, the transcriptional activator produced by this pathway [50]. The INM proteins Myorg/Net37 and Plpp7/Net39 modulate myogenesis through binding to signaling factors (Table 1). Myorg/Net37 promotes myogenesis, likely by enhancing the ER folding and secretion of autocrine myogenic factors [25], whereas Plpp7/Net39 inhibits myogenesis, apparently by binding to mTOR [26].

Lamins themselves have been found to bind and sequester numerous transcriptional activators to limit their activity [51–53]. This mechanism is illustrated by studies of c-Fos, which is associated with lamins A/C in an inactive form in serum-starved cells [51]. Serum induces the activation and nuclear import of ERK1/2, which phosphorylates c-Fos and releases it from lamins. This allows c-Fos to assemble into AP-1 at gene promoters to activate cell proliferation. In this situation, lamins A/C provide both a scaffold for the inactive form of c-Fos, and a platform for its signaling-mediated activation. Conceivably, the NL also might promote the subsequent dephosphorylation and inactivation of intranuclear ERK. A recent study describes a fascinating variation of this theme involving progerin, a mutated form of lamin A that causes HGPS. In a gain-of-function mechanism, progerin appears to sequester and thereby inactivate NRF2 [7^{**}], a critical transcription factor for the antioxidant response. Loss of NRF2 activity in HGPS cells can explain many of the aging-related phenotypes in these models. Other laminopathy mutations, however, can abnormally activate the NRF2 pathway and cause reductive stress [54].

In a distinct mechanism for restriction of gene expression, some NL proteins including lamins A/C and LBR promote the formation and/or maintenance of transcriptionally inactive heterochromatin at the nuclear periphery [16^{**}]. NL proteins accomplish this by selective interactions with lamina-associated chromatin domains (LADs) [55]. Some NL proteins also bind transcriptional repressors, which can help to reinforce a heterochromatic environment at the NL [55]. Disruption of heterochromatin interactions at the NL can cause substantial

changes in gene expression, which in turn can indirectly activate new signaling pathways [55,56].

Signaling control by modulation of actin assembly and NL composition

Recent studies have revealed intriguing mechanisms that link the NL to signaling through emerin-mediated nucleation of F-actin assembly [57]. One system [58**] involves the transcription factor MKL-1, which is imported into the nucleus to activate the expression of motility genes after cell stimulation. The MKL-1 response is inhibited by intranuclear G-actin, which forms a complex with MKL-1 and mediates its rapid nuclear export [59]. MKL-1-mediated transcription is defective in lamin A-null cells or in cells expressing the lamin A (N195K) disease mutant due to the redistribution of emerin from the INM to the peripheral ER [58**]. The actin binding activity of emerin is required for the MKL-1 response and can explain these effects. By promoting intranuclear actin polymerization, INM-localized emerin would correspondingly decrease the local concentration of G-actin and thereby favor the nuclear retention (and transcriptional activity) of MKL-1.

Another regulatory role for emerin involving actin assembly was revealed in studies of Polycomb-mediated gene repression in epidermal precursor cells subjected to mechanical strain [32**]. Repression by Polycomb in this model depends on the depletion of intranuclear actin, which limits actin-stimulated chromatin remodeling and RNA polymerase [59]. Nuclear actin reduction results from increased actin polymerization in the cytoplasm mediated by emerin, which becomes partially relocated to the ONM and peripheral ER by the mechanical forces [32**]. The mechanism for emerin redistribution in these cells is unknown, but might involve posttranslational modifications that decrease binding to lamins or increase binding to immobile ER sites.

The above studies provide examples of how changes in the composition of the NL (i.e., by redistribution of emerin to the peripheral ER) can regulate signaling. This theme also is apparent in studies of the stretch-induced proliferative response of vascular smooth muscle cells, which requires a reduction in the levels of lamins A/C and emerin [60*]. Thus, modulation of NL composition as a means of affecting signaling output can occur in diverse physiological circumstances.

Pleiotropic signaling defects associated with disruption of NL proteins

Discrete NL mutations can lead to aberrations in multiple signaling and gene expression pathways in cells and animal tissues. This is exemplified with a mouse model containing a knock-in mutation in lamin A (H222P) that causes cardiomyopathy [5,61]. Analysis of heart from these animals reveals elevated activation of all three major MAP kinases [5,62], as well as AKT/mTOR [6] and TGF- β /Smad [63*], and decreased activation of Wnt/ β -catenin [49] (Table 1). Recent studies indicate that ERK activation in this model is promoted by the elevated TGF- β /Smad signaling [63*] and thus may be a downstream effect. Whether the increased TGF- β signaling is connected to MAN1 is unknown. However, elevation of MAP kinase activity without increased TGF- β is observed in cultured cell and mouse models involving depletion of Lem2 [64,65]. Extensive crosstalk between the MAP kinase and

AKT/mTOR pathways has been documented [66], and might contribute to co-activation of these pathways in the lamin A (H222P) mouse [9,62]. Pleiotropic signaling effects also are seen in cultured cell and mouse models for HGPS, which reveal increased Notch [67] and NF- κ B [8] signaling, and decreased Wnt signaling [68]. The elevated NF- κ B is an indirect result of an increased DNA damage response resulting from genomic instability [8], but causes of the other signaling defects are unknown. It is challenging to distinguish primary from secondary effects in these models, since reciprocal signaling pathway crosstalk [63[•]] and changes in the abundance of signaling factors or NL proteins can occur.

Conclusion

Recent work has emphasized that the NL, in addition to providing a structural scaffold at the nuclear periphery, also regulates numerous signaling factors that mediate transcriptional responses. At the present time, a cardinal challenge is to obtain deeper mechanistic understanding of how various signaling pathways are connected to individual NL proteins, and whether these connections are direct or indirect. Recent precedents suggest that modulation of NL composition or structure by mechanical cues or other mechanisms [32^{••}, 60[•], 69, 70] could have widespread regulatory significance. Moreover, the nanoscale organization of the NL in different cell types, including the composition of complexes with different transmembrane proteins and lamins, is likely to be important. Regulatory input also might come from low abundance proteins not yet identified as NL residents, or from the distinctive lipid signaling that occurs at the INM and within the nucleus [71, 72, 73[•]]. Some of the diseases caused by mutations in the genes for NL-associated transmembrane proteins are very similar to those caused by mutations in lamin A [3], emphasizing the structural and functional integration of the NL. This supports the value of a system-wide perspective for understanding this fascinating cellular structure.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Burke B, Stewart CL. The nuclear lamins: flexibility in function. *Nat Rev Mol Cell Biol.* 2013; 14:13–24. [PubMed: 23212477]
2. Gruenbaum Y, Foisner R. Lamins: nuclear intermediate filament proteins with fundamental functions in nuclear mechanics and genome regulation. *Annu Rev Biochem.* 2015; 84:131–164. [PubMed: 25747401]
3. Worman HJ, Ostlund C, Wang YX. Diseases of the nuclear envelope. *Cold Spring Harb Perspect Biol.* 2010; 2:a000760. [PubMed: 20182615]
4. Boone PM, Yuan B, Gu S, Ma ZW, Gambin T, Gonzaga-Jauregui C, Jain M, Murdock TJ, White JJ, Jhangiani SN, et al. Hutterite-type cataract maps to chromosome 6p21.32-p21.31, cosegregates with

a homozygous mutation in LEMD2, and is associated with sudden cardiac death. *Mol Genet Genom Med*. 2016; 4:77–94.

5. Choi JC, Worman HJ. Nuclear envelope regulation of signaling cascades. *Adv Exp Med Biol*. 2014; 773:187–206. [PubMed: 24563349]
6. Choi JC, Muchir A, Wu W, Iwata S, Homma S, Morrow JP, Worman HJ. Temsirolimus activates autophagy and ameliorates cardiomyopathy caused by lamin A/C gene mutation. *Sci Transl Med*. 2012; 4:ra102.
- 7••. Kubben N, Zhang WQ, Wang LX, Voss TC, Yang JP, Qu J, Liu GH, Misteli T. Repression of the antioxidant NRF2 pathway in premature aging. *Cell*. 2016; 165:1361–1374. The authors provide strong evidence that the premature aging phenotype in HGPS cell models involves suppression of NRF2, a central transcriptional factor in the antioxidant response. Mechanistically, this was linked to intranuclear sequestration of NRF2 by progerin, the mutated form of lamin A in HGPS. Reactivation of the NRF2 pathway by pharmacological or genetic means reversed the phenotypes in HGPS cells. [PubMed: 27259148]
8. Osorio FG, Barcena C, Soria-Valles C, Ramsay AJ, de Carlos F, Cobo J, Fueyo A, Freije JM, Lopez-Otin C. Nuclear lamina defects cause ATM-dependent NF-kappaB activation and link accelerated aging to a systemic inflammatory response. *Genes Dev*. 2012; 26:2311–2324. [PubMed: 23019125]
9. Ramos FJ, Chen SC, Garelick MG, Dai DF, Liao CY, Schreiber KH, MacKay VL, An EH, Strong R, Ladiges WC, et al. Rapamycin reverses elevated mTORC1 signaling in lamin A/C-deficient mice, rescues cardiac and skeletal muscle function, and extends survival. *Sci Transl Med*. 2012; 4:ra103.
10. Stubenvoll A, Rice M, Wietelmann A, Wheeler M, Braun T. Attenuation of Wnt/beta-catenin activity reverses enhanced generation of cardiomyocytes and cardiac defects caused by the loss of emerin. *Hum Mol Genet*. 2015; 24:802–813. [PubMed: 25274778]
11. Shimi T, Kittisopikul M, Tran J, Goldman AE, Adam SA, Zheng YX, Jaqaman K, Goldman RD. Structural organization of nuclear lamins A, C, B1, and B2 revealed by superresolution microscopy. *Mol Biol Cell*. 2015; 26:4075–4086. [PubMed: 26310440]
12. Xie W, Chojnowski A, Boudier T, Lim JSY, Ahmed S, Ser Z, Stewart C, Burke B. A-type lamins form distinct filamentous networks with differential nuclear pore complex associations. *Curr Biol*. 2016; 26:2651–2658. [PubMed: 27641764]
- 13••. Turgay Y, Eibauer M, Goldman AE, Shimi T, Khayat M, Ben-Harush K, Dubrovsky-Gaupp A, Sapra KT, Goldman RD, Medalia O. The molecular architecture of lamins in somatic cells. *Nature*. 2017; 543:261–264. This cryo-EM tomography study with permeabilized, nuclease-treated mammalian cells provides the most informative structural visualization of the NL in a somatic cell up to now. The key finding was that lamin filaments in dividing cells have an ~3.5 nm diameter. Immunogold labeling revealed that A and B-type lamins occur in separate, structurally similar filaments, extending earlier results obtained by superresolution light microscopy. [PubMed: 28241138]
14. Herrmann H, Aebi U. Intermediate filaments: structure and assembly. *Cold Spring Harb Perspect Biol*. 2016; 8:a018242. [PubMed: 27803112]
15. Barton LJ, Soshnev AA, Geyer PK. Networking in the nucleus: a spotlight on LEM-domain proteins. *Curr Opin Cell Biol*. 2015; 34:1–8. [PubMed: 25863918]
- 16••. Solovei I, Wang AS, Thanisch K, Schmidt CS, Krebs S, Zwerger M, Cohen TV, Devys D, Foisner R, Peichl L, et al. LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. *Cell*. 2013; 152:584–598. This landmark paper analyzed the tethering of heterochromatin to the nuclear periphery in mouse models from a developmental perspective. It remains the most comprehensive analysis of the problem in a mammalian organism up to now. [PubMed: 23374351]
17. Thanisch K, Song C, Engelkamp D, Koch J, Wang A, Hallberg E, Foisner R, Leonhardt H, Stewart CL, Joffe B, et al. Nuclear envelope localization of LEMD2 is developmentally dynamic and lamin A/C dependent yet insufficient for heterochromatin tethering. *Differentiation*. 2017; 94:58–70. [PubMed: 28056360]
- 18•. Tsai PL, Zhao CG, Turner E, Schlieker C. The Lamin B receptor is essential for cholesterol synthesis and perturbed by disease-causing mutations. *Elife*. 2016; 5:e16011. LBR was found to be essential for cholesterol biosynthesis in HeLa cells by gene disruption, and its sterol reductase domain was sufficient to restore growth in cholesterol-restricted medium. Knock-in alleles

representing human LBR disease mutations were deficient in cholesterol biosynthesis or unstable, suggesting that the human diseases may be caused by cholesterol deficiency. [PubMed: 27336722]

19. Tapley EC, Starr DA. Connecting the nucleus to the cytoskeleton by SUN-KASH bridges across the nuclear envelope. *Curr Opin Cell Biol.* 2013; 25:57–62. [PubMed: 23149102]
20. Chang W, Worman HJ, Gundersen GG. Accessorizing and anchoring the LINC complex for multifunctionality. *J Cell Biol.* 2015; 208:11–22. [PubMed: 25559183]
21. Thakar K, May CK, Rogers A, Carroll CW. Opposing roles for distinct LINC complexes in regulation of the small GTPase RhoA. *Mol Biol Cell.* 2017; 28:182–191. [PubMed: 28035049]
22. Schirmer EC, Florens L, Guan TL, Yates JR, Gerace L. Nuclear membrane proteins with potential disease links found by subtractive proteomics. *Science.* 2003; 301:1380–1382. [PubMed: 12958361]
23. Wilkie GS, Korfali N, Swanson SK, Malik P, Srsen V, Batrakou DG, Heras JD, Zuleger N, Kerr ARW, Florens L, et al. Several novel nuclear envelope transmembrane proteins identified in skeletal muscle have cytoskeletal associations. *Mol Cell Proteom.* 2011; 10:M110.003129.
24. Korfali N, Wilkie GS, Swanson SK, Srsen V, Batrakou DG, Fairley EAL, Malik P, Zuleger N, Goncharevich A, Heras JDL, et al. The leukocyte nuclear envelope proteome varies with cell activation and contains novel transmembrane proteins that affect genome architecture. *Mol Cell Proteom.* 2010; 9:2571–2585.
25. Datta K, Guan T, Gerace L. NET37, a nuclear envelope transmembrane protein with glycosidase homology, is involved in myoblast differentiation. *J Biol Chem.* 2009; 284:29666–29676. [PubMed: 19706595]
26. Liu GH, Guan T, Datta K, Coppinger J, Yates J, Gerace L. Regulation of myoblast differentiation by the nuclear envelope protein NET39. *Mol Cell Biol.* 2009; 29:5800–5812. [PubMed: 19704009]
27. Mudumbi KC, Schirmer EC, Yang W. Single-point single-molecule FRAP distinguishes inner and outer nuclear membrane protein distribution. *Nat Commun.* 2016; 7:12562. [PubMed: 27558844]
- 28•. Smoyer CJ, Katta SS, Gardner JM, Stoltz L, McCroskey S, Bradford WD, McClain M, Smith SE, Slaughter BD, Unruh JR, et al. Analysis of membrane proteins localizing to the inner nuclear envelope in living cells. *J Cell Biol.* 2016; 215:575–590. A split-GFP approach was used to screen over 1000 predicted and known transmembrane proteins in *S. cerevisiae* for their ability to access the INM. Nearly half of the proteins had this capacity, even though most were not concentrated at the INM. The data support a diffusion-retention model for transmembrane protein localization to the INM. [PubMed: 27831485]
29. Ungricht R, Kutay U. Establishment of NE asymmetry —targeting of membrane proteins to the inner nuclear membrane. *Curr Opin Cell Biol.* 2015; 34:135–141. [PubMed: 26112002]
30. Malik P, Korfali N, Srsen V, Lazou V, Batrakou DG, Zuleger N, Kavanagh DM, Wilkie GS, Goldberg MW, Schirmer EC. Cell-specific and lamin-dependent targeting of novel transmembrane proteins in the nuclear envelope. *Cell Mol Life Sci.* 2010; 67:1353–1369. [PubMed: 20091084]
31. Mimura Y, Takagi M, Clever M, Imamoto N. ELYS regulates the localization of LBR by modulating its phosphorylation state. *J Cell Sci.* 2016; 129:4200–4212. [PubMed: 27802161]
- 32••. Le HQ, Ghatak S, Yeung CYC, Tellkamp F, Gunschmann C, Dieterich C, Yeroslaviz A, Haberland B, Pombo A, Niessen CM, et al. Mechanical regulation of transcription controls Polycomb-mediated gene silencing during lineage commitment. *Nat Cell Biol.* 2016; 18:864–875. This is one of the first papers to provide mechanistic insight on how cell mechanics can alter NL structure to modulate gene expression. Mechanical strain was found to induce Polycomb-mediated chromatin silencing of lineage-specific genes in epidermal progenitor cells. This process involved cytoplasmic actin polymerization coordinated by nonmuscle myosin IIA and emerin that had become relocalized to the ONM and peripheral ER. [PubMed: 27398909]
33. Wheeler MA, Warley A, Roberts RG, Ehler E, Ellis JA. Identification of an emerin-beta-catenin complex in the heart important for intercalated disc architecture and beta-catenin localisation. *Cell Mol Life Sci.* 2010; 67:781–796. [PubMed: 19997769]
- 34••. Saunders CA, Harris NJ, Willey PT, Woolums BM, Wang Y, McQuown AJ, Schoenhofen A, Worman HJ, Dauer WT, Gundersen GG, et al. TorsinA controls TAN line assembly and the

retrograde flow of dorsal perinuclear actin cables during rearward nuclear movement. *J Cell Biol.* 2017; 216:657–674. The paper decisively establishes that TorsinA and its activator LAP1 are required for rearward nuclear movement in migrating fibroblasts. The proteins are needed for formation of the nesprin-2-containing ‘TAN lines’ on the surface of the NE that connect to perinuclear actin cables undergoing retrograde flow, and for the retrograde actin flow itself. [PubMed: 28242745]

- 35•. Demircioglu FE, Sosa BA, Ingram J, Ploegh HL, Schwartz TU. Structures of TorsinA and its disease-mutant complexed with an activator reveal the molecular basis for primary dystonia. *Elife.* 2016; 5:e17983. This paper confirmed and extended the modeling from the preceding paper (Ref. [37**]) by providing atomic structures of the luminal domain of a LAP1 paralog, LULL1, co-crystallized with TorsinA and with a disease-associated form of TorsinA (*E*). [PubMed: 27490483]
36. Lauder milch E, Schlieker C. Torsin ATPases: structural insights and functional perspectives. *Curr Opin Cell Biol.* 2016; 40:1–7. [PubMed: 26803745]
- 37•. Sosa BA, Demircioglu FE, Chen JZ, Ingram J, Ploegh HL, Schwartz TU. How lamin-associated polypeptide 1 (LAP1) activates Torsin. *Elife.* 2014;3. The atomic structure of the luminal domain of LAP1 revealed an atypical AAA+ ATPase fold. Molecular modeling and mutational analysis indicated that LAP1 possesses an ‘arginine finger’ lacking in TorsinA that activates the latter, and that the enzymatically active form of TorsinA is a LAP1-TorsinA complex.
- 38•. Grillet M, Dominguez Gonzalez B, Sicart A, Pottler M, Cascalho A, Billion K, Hernandez Diaz S, Swerts J, Naismith TV, Goukko NV, et al. Torsins are essential regulators of cellular lipid metabolism. *Dev Cell.* 2016; 38:235–247. A knockout of TorsinA in *Drosophila* resulted in lethality. This was traced to defects in the fat body, which showed elevated triglyceride synthesis and reduced phosphoglyceride synthesis. Although the direct target of TorsinA was not determined, it might be NE-linked regulators including the phosphatidic acid phosphatase lipin, or the INM phosphatase (Ctdnep1) that activates lipin. [PubMed: 27453503]
39. Cho S, Irianto J, Discher DE. Mechanosensing by the nucleus: from pathways to scaling relationships. *J Cell Biol.* 2017; 216:305–315. [PubMed: 28043971]
40. Isermann P, Lammerding J. Nuclear mechanics and mechanotransduction in health and disease. *Curr Biol.* 2013; 23:R1113–R1121. [PubMed: 24355792]
41. Buxboim A, Swift J, Irianto J, Spinler KR, Dingal PCDP, Athirasala A, Kao YRC, Cho S, Harada T, Shin JW, et al. Matrix elasticity regulates lamin-A, C phosphorylation and turnover with feedback to actomyosin. *Curr Biol.* 2014; 24:1909–1917. [PubMed: 25127216]
- 42•. Guilluy C, Osborne LD, Van Landeghem L, Sharek L, Superfine R, Garcia-Mata R, Burridge K. Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. *Nat Cell Biol.* 2014; 16:376–381. This study made the remarkable observation that repeated force application to isolated nuclei, using beads coated with antibodies to nesprin and laser tweezers, caused the nuclear lamina to stiffen. The effect required emerin phosphorylation by c-Src and augmented lamin A association with nesprin. A role for this mechanism in mechanotransduction in cells was suggested. [PubMed: 24609268]
43. Lin F, Morrison JM, Wu W, Worman HJ. MAN1, an integral protein of the inner nuclear membrane, binds Smad2 and Smad3 and antagonizes transforming growth factor-beta signaling. *Hum Mol Genet.* 2005; 14:437–445. [PubMed: 15601644]
44. Pan D, Estevez-Salmeron LD, Stroschein SL, Zhu XL, He J, Zhou SL, Luo KX. The integral inner nuclear membrane protein MAN1 physically interacts with the R-Smad proteins to repress signaling by the transforming growth factor-beta superfamily of cytokines. *J Biol Chem.* 2005; 280:15992–16001. [PubMed: 15647271]
45. Osada SI, Ohmori S, Taira M. XMAN1, an inner nuclear membrane protein, antagonizes BMP signaling by interacting with Smad1 in *Xenopus* embryos. *Development.* 2003; 130:1783–1794. [PubMed: 12642484]
46. Raju GP, Dimova N, Klein PS, Huang HC. SANE, a novel LEM domain protein, regulates bone morphogenetic protein signaling through interaction with Smad1. *J Biol Chem.* 2003; 278:428–437. [PubMed: 12393873]
47. Bourgeois B, Gilquin B, Tellier-Lebegue C, Ostlund C, Wu W, Perez J, El Hage P, Lallemand F, Worman HJ, Zinn-Justin S. Inhibition of TGF-beta Signaling at the Nuclear Envelope:

- Characterization of Interactions Between MAN1, Smad2 and Smad3, and PPM1A. *Sci Signal*. 2013; 6:ra49. [PubMed: 23779087]
48. Markiewicz E, Tilgner K, Barker N, van de Wetering M, Clevers H, Dorobek M, Hausmanowa-Petrusewicz I, Ramaekers FCS, Broers JLV, Blankesteyn WM, et al. The inner nuclear membrane protein emerin regulates beta-catenin activity by restricting its accumulation in the nucleus. *EMBO J*. 2006; 25:3275–3285. [PubMed: 16858403]
 49. Le Dour C, Macquart C, Sera F, Homma S, Bonne G, Morrow JP, Worman HJ, Muchir A. Decreased WNT/beta-catenin signalling contributes to the pathogenesis of dilated cardiomyopathy caused by mutations in the lamin A/C gene. *Hum Mol Genet*. 2017; 26:333–343. [PubMed: 28069793]
 50. Lee B, Lee TH, Shim J. Emerin suppresses Notch signaling by restricting the Notch intracellular domain to the nuclear membrane. *Biochim Biophys Acta Mol Cell Res*. 2017; 1864:303–313.
 51. Gonzalez JM, Navarro-Puche A, Casar B, Crespo P, Andres V. Fast regulation of AP-1 activity through interaction of lamin A/C, ERK1/2, and c-Fos at the nuclear envelope. *J Cell Biol*. 2008; 183:653–666. [PubMed: 19015316]
 52. Malhas AN, Lee CF, Vaux DJ. Lamin B1 controls oxidative stress responses via Oct-1. *J Cell Biol*. 2009; 184:45–55. [PubMed: 19139261]
 53. Naetar N, Ferraioli S, Foisner R. Lamins in the nuclear interior—life outside the lamina. *J Cell Sci*. 2017; 130:2087–2096. [PubMed: 28668931]
 54. Dialynas G, Shrestha OK, Ponce JM, Zwerger M, Thiemann DA, Young GH, Moore SA, Yu LP, Lammerding J, Wallrath LL. Myopathic lamin mutations cause reductive stress and activate the Nrf2/Keap-1 pathway. *PLoS Genet*. 2015:11.
 55. van Steensel B, Belmont AS. Lamina-associated domains: links with chromosome architecture, heterochromatin, and gene repression. *Cell*. 2017; 169:780–791. [PubMed: 28525751]
 56. Chen HY, Zheng XB, Zheng YX. Age-associated loss of lamin-B leads to systemic inflammation and gut hyperplasia. *Cell*. 2014; 159:829–843. The study revealed how changes in NL composition (specifically, depletion of lamin B) in the fat body of aging *Drosophila* alters heterochromatin structure and gene expression programs. The lamin loss resulted in secretion of inflammatory cytokines that impaired gut homeostasis. [PubMed: 25417159]
 57. Holaska JM, Kowalski AK, Wilson KL. Emerin caps the pointed end of actin filaments: evidence for an actin cortical network at the nuclear inner membrane. *PLoS Biol*. 2004; 2:1354–1362.
 58. Ho CY, Jaalouk DE, Vartiainen MK, Lammerding J. Lamin A/C and emerin regulate MKL1-SRF activity by modulating actin dynamics. *Nature*. 2013; 497:507–511. The analysis described how cardiomyopathy-causing disease mutations in the genes for lamins A/C and emerin can blunt the serum response factor (SRF) program, which may underlie disease progression. The data support that model that emerin causes intranuclear actin depletion to promote SRF activity. [PubMed: 23644458]
 59. Virtanen JA, Vartiainen MK. Diverse functions for different forms of nuclear actin. *Curr Opin Cell Biol*. 2017; 46:33–38. [PubMed: 28092729]
 60. Qi YX, Yao QP, Huang K, Shi Q, Zhang P, Wang GL, Han Y, Bao H, Wang L, Li HP, et al. Nuclear envelope proteins modulate proliferation of vascular smooth muscle cells during cyclic stretch application. *Proc Natl Acad Sci U S A*. 2016; 113:5293–5298. The authors found that force-induced proliferation of vascular smooth muscle cells was accompanied by the reduction in lamin A and emerin. Ectopic expression of these proteins diminished proliferation, whereas RNAi-mediated knockdown promoted proliferation, linking regulation of lamin A and emerin levels to cell proliferation in this model. [PubMed: 27114541]
 61. Arimura T, Helbling-Leclerc A, Varnous S, Niel F, Lacene E, Fromes Y, Toussaint M, Mura AM, Keller DI, Amthor H, et al. Mouse model carrying H222P-Lmna mutation develops muscular dystrophy and dilated cardiomyopathy similar to human striated muscle laminopathies. *Hum Mol Genet*. 2005; 14:155–169. [PubMed: 15548545]
 62. Muchir A, Pavlidis P, Decostre V, Herron AJ, Arimura T, Bonne G, Worman HJ. Activation of MAPK pathways links LMNA mutations to cardiomyopathy in Emery-Dreifuss muscular dystrophy. *J Clin Invest*. 2007; 117:1282–1293. [PubMed: 17446932]

- 63•. Chatzifrangkeskou M, Le Dour C, Wu W, Morrow JP, Joseph LC, Beuvin M, Sera F, Homma S, Vignier N, Mougenot N, et al. ERK1/2 directly acts on CTGF/CCN2 expression to mediate myocardial fibrosis in cardiomyopathy caused by mutations in the lamin A/C gene. *Hum Mol Genet.* 2016; 25:2220–2233. This paper revealed that cardiomyopathy caused by the lamin A (H222P) mutation involves elevated TGF- β signaling. Inhibitor studies suggested that elevated TGF- β was upstream of ERK activation and the secretion of connective tissue growth factor, two other pathways that contribute to disease pathogenesis. [PubMed: 27131347]
64. Huber MD, Guan T, Gerace L. Overlapping functions of nuclear envelope proteins NET25 (Lem2) and emerin in regulation of extracellular signal-regulated kinase signaling in myoblast differentiation. *Mol Cell Biol.* 2009; 29:5718–5728. [PubMed: 19720741]
65. Tapia O, Fong LG, Huber MD, Young SG, Gerace L. Nuclear envelope protein Lem2 is required for mouse development and regulates MAP and AKT Kinases. *PLOS ONE.* 2015; 10:e0116196. [PubMed: 25790465]
66. Mendoza MC, Er EE, Blenis J. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends Biochem Sci.* 2011; 36:320–328. [PubMed: 21531565]
67. Scaffidi P, Misteli T. Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. *Nat Cell Biol.* 2008; 10:452–459. [PubMed: 18311132]
68. Hernandez L, Roux KJ, Wong ES, Mounkes LC, Mitalif R, Navasankari R, Rai B, Cool S, Jeong JW, Wang H, et al. Functional coupling between the extracellular matrix and nuclear lamina by Wnt signaling in progeria. *Dev Cell.* 2010; 19:413–425. [PubMed: 20833363]
69. Webster BM, Lusk CP. Border safety: quality control at the nuclear envelope. *Trends Cell Biol.* 2016; 26:29–39. [PubMed: 26437591]
70. Khmelinskii A, Blaszczyk E, Pantazopoulou M, Fischer B, Omnis DJ, Le Dez G, Brossard A, Gunnarsson A, Barry JD, Meurer M, et al. Protein quality control at the inner nuclear membrane. *Nature.* 2014; 516:410–413. [PubMed: 25519137]
71. Bahmanyar S. Spatial regulation of phospholipid synthesis within the nuclear envelope domain of the endoplasmic reticulum. *Nucleus.* 2015; 6:102–106. [PubMed: 25671705]
72. Barlow CA, Laishram RS, Anderson RA. Nuclear phosphoinositides: a signaling enigma wrapped in a compartmental conundrum. *Trends Cell Biol.* 2010; 20:25–35. [PubMed: 19846310]
- 73•. Enyedi B, Jelcic M, Niethammer P. The cell nucleus serves as a mechanotransducer of tissue damage-induced inflammation. *Cell.* 2016; 165:1160–1170. One of the phospholipase A2 enzymes involved in production of pro-inflammatory eicosanoids is localized to the nucleus. This study showed that the enzyme becomes activated by hypotonic swelling of cells, by a process that involves its targeting to the INM. The authors suggest that this results from swelling-induced expansion of the INM bilayer. [PubMed: 27203112]
74. Muchir A, Wu W, Choi JC, Iwata S, Morrow J, Homma S, Worman HJ. Abnormal p38 mitogen-activated protein kinase signaling in dilated cardiomyopathy caused by lamin A/C gene mutation. *Hum Mol Genet.* 2012; 21:4325–4333. [PubMed: 22773734]
75. Le Dour C, Wu W, Bereziat V, Capeau J, Vigouroux C, Worman HJ. Extracellular matrix remodeling and transforming growth factor-beta signaling abnormalities induced by lamin A/C variants that cause lipodystrophy. *J Lipid Res.* 2017; 58:151–163. [PubMed: 27845687]
76. Cohen TV, Kosti O, Stewart CL. The nuclear envelope protein MAN1 regulates TGF beta signaling and vasculogenesis in the embryonic yolk sac. *Development.* 2007; 134:1385–1395. [PubMed: 17329363]
77. Ishimura A, Ng JK, Taira M, Young SG, Osada SI. Man1, an inner nuclear membrane protein, regulates vascular remodeling by modulating transforming growth factor beta signaling. *Development.* 2006; 133:3919–3928. [PubMed: 16943282]
78. Muchir A, Pavlidis P, Bonne G, Hayashi YK, Worman HJ. Activation of MAPK in hearts of EMD null mice: similarities between mouse models of X-linked and autosomal dominant Emery-Dreifuss muscular dystrophy. *Hum Mol Genet.* 2007; 16:1884–1895. [PubMed: 17567779]
79. Zhou C, Li C, Zhou B, Sun HQ, Koullourou V, Holt I, Puckelwartz MJ, Warren DT, Hayward R, Lin ZY, et al. Novel nesprin-1 mutations associated with dilated cardiomyopathy cause nuclear envelope disruption and defects in myogenesis. *Hum Mol Genet.* 2017; 26:2258–2276. [PubMed: 28398466]

Figure 1.

Model of nuclear envelope organization in mammalian cells. The diagram illustrates the direct connection of the ONM with the peripheral ER, and the lipid bilayer continuity between the ONM and INM at NPCs. Nuclear lamin filaments [13**] are depicted as a green meshwork underlying the INM. Widely studied INM proteins are shown, with transmembrane segments and folded domains represented by cylinders spanning the lipid bilayer and external to it, respectively. The LEM domain, an ~45 amino acid segment near the N-terminus of LAP2 β , emerin, MAN1 and Lem2, is designated as a short orange cylinder. The INM proteins represented have between 1–10 transmembrane segments, and all contain a nucleoplasmic region of at least 200 amino acids that is predicted to contain substantial intrinsic disorder (depicted as an extended thread). Torsin1A has a membrane-embedded segment but is not strictly a transmembrane protein. The luminal domains of LAP1 and TorsinA each have an AAA+ related fold that associates to form heterooligomers [35*]. The LINC complex comprises SUN domain proteins spanning the INM associated with nesprins spanning the ONM [19,20]. The major SUN domain proteins of somatic cells (Sun1 and Sun2) each form a trimeric assembly via their luminal SUN domains. Nesprins, which occur as many splice isoforms, all contain a conserved ~30 amino acid luminal region that interacts with the SUN domain trimers. The cytoplasmic domains of nesprin-1 and nesprin-2 contain many tandem spectrin repeats (depicted as discs) and interact with actin filaments and microtubules by a variety of linker molecules (not shown). Nesprin-3 associates with intermediate filaments via the plectin linker [19,20]. Chromatin is associated with the INM by interaction with LBR and LEM domain proteins.

Signaling aberrations linked to NL proteins. KI, knockin; KO, knockout; KD, knockdown; TG, transgenic; ND, not determined

Table 1

NL protein	Signaling pathway	Perturbation/model	Signaling effect	Interacting factor	References
Lamin A	MAP kinases (ERK, JNK, p38)	Lamin A (H222P), KI mouse	Increased	ND	[62,63,74]
	TGF- β	Lamin A (H222P) KI, mouse; Lamin A (R482Q) TG, mouse	Increased	Smad 2/3	[63,75]
	Wnt	Lamin A (H222P) KI, mouse; Lamin A (H530P) KI, mouse	Decreased	ND	[49,68]
	AKT/mTOR	Lamin A (H222P) KI, mouse; Lamin A KO, mouse	Increased	ND	[6,9]
	Notch	Ectopic progerin, cultured cells	Increased	ND	[67]
	NF- κ B	Lamin A (G609G) KI, mouse; Zmpste24 KO, mouse	Increased	ND	[8]
	Proliferation response	Lamin A KD & KO, cultured cells	Increased	ERK, c-Fos	[51]
	Antioxidant response	Ectopic progerin, cultured cells	Decreased	NRF2	[7*]
	Antioxidant response	Mutated lamin C TG, <i>Drosophila</i>	Increased	ND	[54]
Lamin A, Emerin	Serum response factor	Lamin A (N195Q) cultured cells; Lamin A & Emerin KO, cultured cells,	Decreased	Actin? (via Emerin)	[58**]
MAN1	BMP	Ectopic MAN1, <i>Xenopus</i> embryos & cultured cells	Decreased	Smad1	[45,46]
	TGF- β	MAN1 KD, cultured cells; MAN1 KO, mouse	Increased	Smad 2/3	[43,44,76,77]
Emerin	Wnt	Emerin KD, cultured cells; Emerin KO, mouse	Increased	β -Catenin	[10,48]
	MAP kinases (ERK, JNK, p38)	Emerin KD & KO, cultured cells; Emerin KO, mouse	Increased	ND	[78]
	Notch	Emerin KD, cultured cells	Increased	Notch ICD	[50]
	Mechanically induced gene repression	Emerin KD, cultured cells	Decreased	Actin?	[32**]
Lem2	MAP kinases (ERK, JNK, p38)	Lem2 KD, cultured cells; Lem2 KO, mouse	Increased	ND	[64,65]
Nesprin-1	ERK	Nesprin-1 mutants, cultured cells; Nesprin-1 KO, mouse	Increased	ND	[79]
Plpp7/Net39	AKT/mTOR	Net39 KD, cultured cells	Increased	ND	[26]
Myorg/Net37	IGF-1	Net37 KD, cultured cells	Decreased	ND	[25]
Lamin B1	Antioxidant response	Lamin B1 KO, cultured cells	Decreased	ND	[52]
Lamin B	Inflammatory gene expression	Lamin B KD, <i>Drosophila</i>	Increased	ND	[56*]
TorsinA	lipogenesis	TorsinA KO & KD, <i>Drosophila</i> & cultured cells	Triglyceride up, phospholipid down	ND	[38*]
LAP1, TorsinA	Polarized cell migration	LAP1 & TorsinA KD, cultured cells	Decreased	ND	[34**]