Insight into the functional organization of nuclear lamins in health and disease
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Lamins are the main component of the nuclear lamina, a protein meshwork at the inner nuclear membrane which primarily provide mechanical stability to the nucleus. Lamins, type V intermediate filament proteins, are also involved in many nuclear activities. Structural analysis of nuclei revealed that lamins form 3.5 nm thick filaments often interact with nuclear pore complexes. Mutations in the LMNA gene, encoding A-type lamins, have been associated with at least 15 distinct diseases collectively termed laminopathies, including muscle, metabolic and neurological disorders, and premature aging syndrome. It is unclear how laminopath mutations lead to such a wide array of diseases, essentially affecting almost all tissues.

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Introduction
Cells endure mechanical cues, such as shear stress, compression, differential tissue rigidity and strain [1]. The cytoplasm induces forces onto the nucleus wherein the nuclear lamina adjusts the mechanical properties of the nucleus together with other elements to protect chromatin [1–3]. The nuclear lamina consists of a layer of filamentous lamins, together with binding partners, at the nuclear phase of the inner nuclear membrane (INM). The nuclear lamina also plays a role in tethering heterochromatin to the nuclear periphery, maintaining genomic stability, genome organization, and duplication [4,5,7]. Furthermore, together with their binding partners, the lamina is also involved in other cellular functions such as DNA repair, nuclear assembly/disassembly, and gene expression [8,9,10**].

In most vertebrates, four lamin genes, LMNA, LMNB1, LMNB2 and LIII encode five major and three minor types of lamin proteins. The LIII gene has however been lost in mammals [11]. The major mammalian lamins are lamin B1, B2, A and G, the latter being a splice variant of lamin A. While B-type lamins are present in all types of mammalian cells during their development, the expression of A-type lamins increase dramatically at the start of differentiation, except in the lower layer of the epidermis [12] and within the central nervous system [13].

Based on sequence homology, lamins are classified as intermediate filament (IF) proteins and are major components of the cell cytoskeleton [14]. Lamin proteins are composed of an N-terminal head domain, a long coiled-coil central rod domain, a globular C-terminal tail with a nuclear localization signal (NLS), and an immunoglobulin (Ig) fold including a CaaX (C, cysteine; a, aliphatic residue; X, any residue) motif for lamin A and B-type lamins [15]. Post-translational modifications of the CaaX motif include farnesylation on the cysteine residue by a farnesyltransferase; followed by a cleavage of the -aX by a metalloprotease (Zmpste24 or endoprotease RAS converting enzyme 1 (RCE1); and carboxy-methylation of the farnesylated cysteine by isoprenylcysteine carboxyl methyltransferase [16]. Maturation of lamin A requires an additional cleavage step to remove 15 amino acids from the C-terminus along with the farnesylated and carboxy-methylated residues [17]. Thus, only B-type lamins remain farnesylated, and are therefore suggested to retain their interaction with the INM. Further post-translational modifications of mature lamins include multiple phosphorylations [18]. A-type lamin is more soluble than other lamins during cell division and has a mobile low-assembly state in the nucleoplasm [19] which is different from the structured meshwork at the nuclear periphery (Figure 1 [20**]). Recent studies show that a low assembly state of lamin at the nucleoplasm has a role in spatial chromatin organization and may be involved in mechanosignaling [21,22*].

Lamins interact with numerous lamin-binding partners to exert their functions
Lamins have over 100 putative binding proteins whose functions vary from the reciprocal transfer of mechanical information between nucleus and cytoplasm, to cell migration, and cell differentiation [23]. Lamin-binding partners can be structural proteins, for example, LAP2α [24], nesprin [25], and SUN proteins [26], signaling...
molecules (via LEM proteins) or transcription factors (pRb, ERK1/2, c-Fos, and SREBP) [14].

The nuclear lamina is connected to the cytoplasmic cytoskeleton via transmembrane Linker of the Nucleoskeleton to the Cytoskeleton (LINC) complexes which are composed of nesprins and SUN (Sad1p, UNC-84) proteins [27]. Lamins interact with SUN proteins at the nuclear periphery where SUN proteins are connected to KASH (Klarsicht, ANC-1, Syne Homology) domain proteins (C-terminal transmembrane domain of nesprins) in the perinuclear space, and where additional nesprin proteins interact with actin or microtubules at the cytoplasmic face of the nuclear membrane (Figure 1) [23,28].

**Structure of lamins in the nuclear lamina**

Since the establishment of cryo-electron tomography (cryo-ET) as a technique that allows *in situ* structural studies to be conducted [29], it’s pivotal role in cell biology [30–33], microbiology [34–36] and virology [37,38] research has been demonstrated. It can depict a particular cellular scene and provide a 3D structural map of an unperturbed, vitrified sample i.e. in a close-to-physiological state [39–41]. Recent developments allow the reconstruction of nuclear structures in unprecedented resolution.

Structural analysis of lamin filaments suggest that lamin proteins form dimers, which further assemble into head-to-tail polymers. These polymers can interact laterally to form lamin protofilaments, which show the characteristic beaded appearance of lamin filaments decorated with Ig-folds. *Caenorhabditis elegans* lamin (Ce-lamin) is a single lamin protein which must fulfill the required functions of both A-type and B-type lamins, and it remains farnesylated [42,43]. By the same token, the negative effects of point mutations to its structural integrity is similar to mammalian lamins [44,45]. To date, only the conditions of Ce-lamin assembly *in vitro* into individual highly stable filaments has been worked out [46–48] and studied by cryo-ET [49]. In addition, the expression of Ce-lamin in *Xenopus* oocytes suggested that the basic structure of lamin filaments is tetrameric in cross-section and composed of two head-to-tail
polymers, in which the protofilament is 4–6 nm in width [48]. These findings were further confirmed by studying unstained C. elegans embryos by means of cryo-ET [50]. The recombinantly expressed mammalian laminas (A-type and B-type) often assemble into para-crystalline fibers, thus filament structures are ideally studied in situ [51]. An insight into the structure of lamin meshwork organizations was acquired by cryo-ET, shown in Figure 2 [20**]. In this study nuclei were subjected to chromatin digestion, exposing the filaments in greater detail. The evidence that observed filaments are indeed lamin filaments was provided by immunogold-labeling of lamin filaments in so-called ‘ghost-nuclei’. This allowed for the elucidation of the structure of the lamin meshwork and revealed the assembly in situ of laminas into 3.5 nm thick filaments. It suggested that laminas form two head-to-tail filaments that laterally assemble in a staggered fashion, in which the globular Ig-folds are found every 20 nm (Figure 2c). This may suggest that cellular components restrict the lateral assembly of laminas to a tetrameric structure [52].

The interactions of nuclear pore complexes (NPCs) with laminas previously demonstrated biochemically [53**], also seem evident within the tomographic maps [20**]. Interestingly, recent cryo-ET data shows that laminas are seen emanating from NPCs, as filaments that end or begin at the NPC, thereby suggesting an anchoring point for the laminas (Figure 2b). Moreover, NPCs are found interacting with filaments which traverse them, namely interactions along a continuous lamin filament (Figure 2b, arrowhead). The interactions of NPCs and laminas was recently analyzed using the BioID approach [53**] and indicated that lamin C rather than lamin A interacts with the NPCs. The smaller Ig-fold of lamin C exhibits less steric hindrance compared to the Ig-fold of lamin A, and therefore is probably able to associate with the NPCs more easily. In cells in which lamin C is not expressed, NPCs were found to be separate from lamin A. A diversity of interactions between laminas and NPCs could suggest that adaptor proteins are also involved in the anchoring of the laminas to the NPCs, thereby expanding the repertoire of contact points (Figure 2b).

Cryo-ET has provided an insight into the structure of lamin filaments, but its restricted field of view does not allow the study of the entire organization of the mammalian nuclear lamina. A complementary approach based on super-resolution microscopy studies has revealed that each of the major mammalian laminas (A, C, B1 and B2) form distinctive separate meshworks [10**,53**]. Computational image analysis unveiled certain meshwork properties, such as lamin edge length (0.432 μm of native lamin A in wild-type cells) and the edge connectivity (four edges per face in wild-type native lamin A). Edges are defined as the filamentous meshwork between two lamin intersection points. Interestingly, compared to lamin A and C, lamin B1 has a higher edge length and a greater edge connectivity. Lamin B1 has more edges per face than lamin B2. Likewise, lamin A has more edge connectivity than lamin C [10**]. Despite the fundamental knowledge on the meshwork properties, individual lamin filaments could not be observed at the 3D-structural illumination microscopy (3D-SIM) resolution (110–130 nm). Using photoactivated localization microscopy (PALM) analysis, resolved N-terminal fused mEOS2-lamins as well as antibody-labeled laminas showed that laminas formed bundles or filamentous structures in segregated and distinct networks [53**]. To date, it remains to be elucidated how these networks are altered in cells expressing various laminopathic mutations.

**The effect of laminopathic mutations on lamin structures and functions**

Over 600 point-mutations of the LMNA gene have been identified which can be directly associated with 15 known diseases, termed laminopathies [54]. Although these mutations spread along the entire gene, several ‘hot spot’ mutations give rise to different diseases in specific tissues. These include adipose, muscular and nerve diseases; or to the early aging of multiple tissues (Figure 3a). Understanding the effects of these hot spot mutations can help to reveal the mechanisms of the various laminopathies. Historically, there are two models that explain laminopathy disease phenotypes: the structural–mechanical model and the gene expression model [55]. The structural–mechanical model is based on the idea that profound changes observed in nuclear morphology can be explained by the disrupted filament formation in severe forms of laminopathies such as mandibuloacral dysplasia (MAD, p.R527C, and p.R527H) [56], Werner syndrome (WRN, p.R133, and p.L140R) [57,58], familial partial lipodystrophy (FPLD, p.R439C) [59] and Hutchinson–Gilford progeria syndrome (HGPS) [60,61]. The gene expression model for these diseases suggests that the mutations do not alter the 3D structure but rather disable the binding sites for lamin A/C biological targets, and hence cause an alteration in the gene expression of A-type laminas (Figure 3b).

Recently, an insight into the mechanism of a lipodystrophy-associated mutation in LMNA; p. R482W was revealed by Oldenburg et al. [62**]. The R482W mutant is thought to have the same structure as wild type lamin A [63] but is found to alter the interaction of LMNA with MIR355 at a position in which the expression of MIR335 and H3K27 acetylation is enhanced dramatically. Enhanced histone 3 acetylation at lysine 27 (H3K27 acetylation) on several enhancer sites creates a positive feedback onto MIR335 transcription. The overexpression of miR-335 (microRNA-335) then blocks differentiation of adipocyte progenitors, creating a phenotype of familial partial lipodystrophy (FPLD2). Normally, lamin A binds to a MIR335 locus, and represses the transcription of miR-
which results in increased H3K27 trimethylation (Figure 3b) [62**].

An intriguing hot spot is R527 in lamin A, in which a mutation causes two lipodystrophies; a muscular dystrophy and a progeroid syndrome. R527 is located at the center of the beta-sheet in the globular Ig-like domain,
Lamin mutations and laminopathies. (a) Schematic view of Lamin proteins shows a head domain (Orange), a coiled-coil rod domain (Blue), an immunoglobulin-like domain (Gold) and an un-structured tail domain (Blue tail). The position of the laminopathic mutations along the mammalian laminas are shown at the lower part, taken from uniprot.org (12.20.2017) entries of human lamin A/C, lamin B1 and lamin B2. Lamins are composed a head domain, a coiled-coil rod domain, an immunoglobulin-like domain and a tail domain with other IFs. Each mutation belongs to a disease illustrated by a different colour. For lamin A, muscular dystrophies are distributed along all the protein domains while lipodystrophies are mostly clustered in the Ig-fold of the C-terminal domain. There are several hot spot mutations that cause different diseases, represented as multiple colored bars in the same amino acid position. (b) A schematic view of a wildtype nucleus (left) is comparison to a diseased lamina which coincide with the Structural-Mechanical model (Right half nucleus). The structural-mechanical model is based on stressed nucleus demonstrations. The number of peripheral lamin A increases while the number of B-type laminas and nucleoplasmic lamin A decreases. The gene expression model (Right) shows MIR335 over-expression triggered by its loss of interaction with the R482W lamin mutant. Increased expression of MIR335 creates a positive feedback loop with a histone, which enhances expression of multiple genes.
and its amide proton contributes to a backbone-backbone hydrogen bond. The backbone hydrogen bonds are known to contribute to the folding and stability of proteins on different energy levels. The microenvironment here is much less polar than at the surface of the protein, and removal of positively charged nitrogen destroys the hydrogen bond, which may then lead to an unstable 3D tertiary structure of the mutant lamin [64,65]. The R527H LMNA mutation is associated with MAD; causes atypical distribution of lamin A/C; and disfigures the nuclear envelope. The less severe phenotypic effects in individuals carrying heterozygous R527H can be explained by a hydrogen bond that putatively forms via its imidazole nitrogen of histidine. Because proline is hydrophobic, a R527P mutation is expected to produce an even more severe abnormal phenotype. R527P is also known for abating the interaction of lamin A with both SUN1 and SUN2, as well as emerin [66–68].

Another interesting hot spot is S573, which is highly conserved amongst eukaryotic species is associated with dilated cardiomyopathy-1A (CMD1A), familial partial lipodystrophy type 2 (FPLD2), mandibulofacial dysplasia type A (MADA) and arthropy (S573L). The substitution of serine 573 in both alleles, with the more substantial hydrophobic residue leucine is not well tolerated in the region. Heterozygous S573L mutations can elicit either cardiomyopathy without lipodystrophy or lipodystrophy without cardiomyopathy [69], implying that secondary genetic and/or environmental factors might facilitate tissue involvement. However, the S573L homozygous LMNA mutation, gives rise to a novel phenotype of arthropy, tendinous calcifications, and progeroid features [70]. The homozygotes have a divergent and more severe phenotype than heterozygotes, which cannot be explained purely by the secondary genetic and/or environmental factors that may hint of a structural alterations of the lamin A protein.

It remains an open question how structural alterations affect gene expression. Is it a balance between both that produces different phenotypes, or do they act independently? Small changes that destabilize the structure of a protein may cause a local or global alteration which can affect the strength of interactions with binding partners. This may result in a cascade of differentiated protein interactions and finally, gene expression alteration. A combination of structural and biochemical studies will be the key to understanding the nature of laminopathies.

Concluding remarks
The nuclear lamina is an essential component of the nuclear periphery, which impacts the structure and function of the cell nucleus. Mutations in laminas change the interactions of binding partners; result in altered gene regulation; modify the structure of the nuclear lamina itself; and may therefore cause laminopathies. The structure of the wild type lamin meshwork has paved the way to studying the structure of lamin meshworks in diseased cells, tissues or even whole organisms, such as C. elegans worms or embryos, in combination with developments in super-resolution microscopy. Its correlation to higher resolution structural analysis is crucial to unveiling the mysteries of the nuclear lamina’s organization. Thus, the atomic structure of laminas will hopefully lead to a better understanding of the nuclear lamina in health and disease. With recent advancements in cryo-ET these fundamental aims are within reach.

Conflict of interest statement
Nothing declared.

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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


Using BioD the authors found that lamin C binds to nuclear pore complexes in higher frequencies than lamin A. Moreover, filamentous lamin structures were resolved using the PALM approach.


The structure of the immunoglobulin-like fold has been determined in completion.


The authors identified the first mutations in the LMNA gene encoding the lamin A/C, to be responsible for the autosomal dominant forms of Emery-Dreifuss muscular dystrophy.