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Filaments assembly of ectopically expressed *Caenorhabditis elegans* lamin within *Xenopus* oocytes

Einat Grossman [a], Idit Dahan [a], Reimer Stick [b], Martin W. Goldberg [c], Yosef Gruenbaum [d], Ohad Medalia [a,e,*]

[a] Department of Life Sciences, Ben Gurion University, Beersheva, Israel
[b] Department of Cell Biology, University of Bremen, Bremen, Germany
[c] School of Biological and Biomedical Sciences, University of Durham, Durham, UK
[d] Department of Genetics, Hebrew University of Jerusalem, Jerusalem, Israel
[e] Department of Biochemistry, University of Zurich, Zurich, Switzerland

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ABSTRACT

Lamins are the major components of the nuclear lamina, a filamentous layer underlying the inner nuclear membrane and attached to the peripheral chromatin. Lamins are required for maintaining nuclear shape and are involved in most nuclear activities. Here, we studied the 3D organization of the nuclear lamina formed upon the expression of *Caenorhabditis elegans* lamin (Ce-lamin) within the nucleus of a *Xenopus laevis* oocyte. We show that Ce-lamin forms an intricate 3D meshwork of 5–6 nm lamin protofilaments. The diverse protofilament interactions and organization may shed light upon the unique mechano-elastic properties of the nuclear lamina scaffold supporting the nuclear envelope. The Q159K Hutchinson–Gilford Progeria Syndrome-linked mutation alters interactions between protofilaments within the lamina, leading to the formation of more bundled arrays of less isotropically-oriented protofilaments. Using this system, we show for the first time the organization of lamin proteins that were translated and assembled within the environment of a living cell.

1. Introduction

Lamin proteins, the major constitutes of the nuclear lamina, form intricate fibrous structures underneath the inner nuclear membrane (INM) (Aebi et al., 1986) as well as an intra-nucleus network (Gruenbaum et al., 2005; Hozak et al., 1995; Krohne, 2004). Lamin fibers interact with chromatin and an assortment of proteins that are required for maintaining the structure of the nucleus and, moreover, participate in a variety of nuclear activities, such as DNA replication, transcription and mitosis (Vlcek and Foisner, 2007). Although the nuclear lamina is involved in most nuclear activities, its primary function is to support the nuclear envelope (NE), allowing force-mediated mechano-transduction. Despite the wealth of knowledge on laminal dynamics and function, the structural organization of the nuclear lamina remains poorly understood.

Lamins are classified as the type V intermediate filament (IF) proteins (Parry et al., 1986). Humans possess three lamin genes, with *LMNB1* and *LMNB2* encoding the B-type lamins, B1 and B2, respectively, and *LMNA* encoding lamins A and C (Stuurman et al., 1998). The nematode *Caenorhabditis elegans* (*C. elegans*) possesses a single lamin gene (*lmn-1*) encoding *C. elegans* lamin (Ce-lamin). While Ce-lamin is classified as a B-type lamin, as it is the only lamin in *C. elegans*, it also presents many characteristics of an A-type lamin. Lamin proteins have a well-defined conserved domain structure consisting of a variable NH_2-terminal globular head domain, a central α-helical rod comprising four coiled-coil domains (1A, 1B, 2A, 2B) separated by linker regions L1, L12, and L2, and a globular COOH-terminal tail domain (Conway and Parry, 1990). The lamin head domain plays an important role in lamin filament assembly regulation. In Ce-lamin, this domain is composed of 48 amino acid residues that are not predicted to fold into a distinct conformation. The coiled-coil domains 1A, 1B, 2A, and 2B are organized around heptad repeats (Quinlan et al., 1985). Coiled-coil domains form rope-like structures, and in lamins, these domains form dimers ~55 nm in length (Stuurman et al., 1998; Ben-Harush et al., 2009). An X-ray crystallography study revealed that the structure of the globular C-terminal domain of lamins resembles the immunoglobulin (Ig) structure (Dhe-Paganon et al., 2002; Krimm et al., 2002). This Ig-fold domain consists of 116 residues folded into a β-sandwich of nine β-strands. The core of this globular domain is formed by hydrophobic residues, with most charged

Abbreviations: Ce-lamin, *Caenorhabditis elegans* lamin; Cryo-ET, cryo-electron tomography; HGPS, Hutchinson–Gilford Progeria Syndrome; INM, inner nuclear membrane; NB, nuclear basket; NE, nuclear envelope; NPC, nuclear pore complex.

* Corresponding author at: Department of Biochemistry, University of Zurich, Zurich, Switzerland.
E-mail address: omedalia@bioc.uzh.ch (O. Medalia).

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residues appearing at the surface of the molecule (Krimm et al., 2002), thereby allowing for interactions with other proteins or DNA. An assembly model of the Ce-lamin protein, based on *in vitro* studies, suggests a hierarchal order of assembly wherein lamins are first dimerized. The dimers polymerize to form a polar head-to-tail chain. Next, lateral assembly of two head-to-tail polymers forms a protofilament. Three or four protofilaments can assemble into 10 nm filaments (Ben-Harush et al., 2009), similarly to cytoplasmic IFs, such as vimentin (Winheim et al., 2011).

Mutations in the human *LMNA* gene affect muscle, adipose, bone, nerve, and skin cells and cause at least 11 different heritable diseases, collectively termed laminopathies (Rankin and Ellard, 2006), ranging from muscular dystrophies, through lipodistrophies and neuropathies, to premature aging diseases. Interestingly, the *LMNA* E145K mutation causes severe lobulation of nuclei, a separation of the A- and B-type lamins, alterations in pericentric heterochromatin, abnormally clustered centromeres and mis-localized telomeres (Taimen et al., 2009). Mutation of the corresponding residue in Ce-lamin, Q159K, shows similar effects and alters the entire animal (Wiesel et al., 2008).

Here, we addressed the structural organization of Ce-lamin filaments in the context of the nuclear envelope, in a lamina-like structure. Ce-lamin assembled into a complex 3D meshwork of 5–6 nm lamin tetrameric protofilaments. The diverse protofilament interactions and organization may explain some of the unique mechano-elastic properties of the nuclear lamina scaffold supporting the NE. Moreover, we found that the Hutchinson–Gilford Progeria Syndrome (HGPS)-associated mutation, Q159K, alters interactions between protofilaments within the lamina, leading to the formation of more bundled yet less isotropic-oriented layers of protofilaments.

2. Materials and methods

2.1. Oocyte isolation and micro-injection

Female *Xenopus laevis* (Daudin) were purchased from NASCO (Fort Atkinson, USA). Oocytes were surgically removed and de-folliculized by collagenase treatment, as described previously (Goldberg et al., 2008). RNA was transcribed *in vitro* (AmpiCap Epicentre Biotechnologies) and was injected into the oocyte cytoplasm (100–320 ng/μl in H_2O 50 nl per oocyte) as described (Stick and Goldberg, 2010). Injected oocytes were incubated for 16–24 h at 18 °C to allow for expression of proteins.

2.2. feSEM image acquisition and processing

SEM sample preparation and acquisition was performed as described (Stick and Goldberg, 2010).

2.3. TEM sample preparation

Oocytes were incubated in low salt buffer (LSB: 10 mM Hepes-KOH, pH 7.4, 1 mM KC1, 0.5 mM $MgCl_2$) for 10–20 min. Nuclei were isolated manually into LSB by puncturing the middle of the animal pole of the oocyte. The nuclei were spontaneously pushed out of the oocytes into the buffer. Nuclei were then transferred using a pipette with cut tip onto a glow-discharged 200-mesh carbon-coated copper grid (Quantifoil, Jena, Germany) where they were allowed to settle and attach by natural adhesion. The NE was then broken open with two fine glass needles and spread on the grid. Nuclear contents were removed by washing the sample with LSB 1–3 times. Home-made 15 nm colloidal gold were added to the sample, followed by freeze-plunging vitrification, as described (Dubochet et al., 1988).

2.4. Cryo-electron tomography

Data were collected using a 300 kV FEI Polara transmission electron microscope equipped with a field-emission gun and a Gatan post-column GIF 2002 energy filter. Tilt series images were collected over an angular range of 60° to −60°, with 1.5°–2° increments. De-focus values ranged from −12 to −8 μm. Projection images (2048 × 2048) were aligned to a common origin using fiducial gold markers, and reconstructed by weighted back-projection, as implemented by the TOM toolbox software package (Nickell et al., 2005). Tomograms were reconstructed with a binning factor to yield a 1.64^3 or 1.28^3 nm^3 voxel size. Surface-rendered visualizations were constructed using the surface-rendering option in AMIRA 5.3 software (ZIB, Visage Imaging).

2.5. Image processing and statistics

For averaging of lamin protofilaments, a total of 155 and 350 sub-frames (32 × 32 pixels) from tomograms of wild type (wt) and Q159K Ce-lamin protofilaments, were selected from tomographic x-y slices and aligned using the single particle procedures of the EM image processing software package (Hegerl, 1996). The power spectrum of the averaged structure was calculated and the relevant frequencies were identified. Consequently, Fourier filters were applied to produce a filtered image with the dominant frequencies. The average thickness of Ce-lamin wt and Q159K protofilaments were measured manually using the measuring tool of the EM package (Hegerl, 1996). The persistence length (λ) of the protofilaments was measured by correlating the contour length (s) to the end-to-end distance of the filament (R) according to the equation $\langle R^2(s)\rangle = 4\,\lambda\, s(1-(2\,\lambda/s)(1-e-s/2\lambda))$ (Wagner et al., 2007). Statistical analysis of protofilaments bundling was performed manually on three tomograms of wt or Q159K Ce-lamin (i.e. a total of six tomograms). Two criteria were essential for defining protofilaments as being bundled. First, that the protofilaments are parallel and second, that the distance between them does not exceed 15 nm.

3. Results

Previously, we studied the supramolecular organization of Ce-lamin proteins using an *in vitro* assembly approach (Ben-Harush et al., 2009). That study revealed that the tetrameric protofilament of Ce-lamin is composed of two staggered head-to-tail polymers. Here, we looked into the assembly of the Ce-lamin in the context of the NE by addressing ectopically expressed Ce-lamin protein in *X. laevis* oocytes and by physically isolating NEs free of chromatin and other adhering material, thereby maintaining the nuclear lamina in place (Aebi et al., 1986).

Xenopus oocytes mainly express lamin B3 (lamin LIII), a B-type lamin (Stick, 1994). This endogenous lamin, namely lamin B3 is hardly detectable using cryo-electron microscopy without the use of detergents (data not shown), presumably, due to its intimate interactions with the INM.

3.1. Scanning electron microscope (SEM) inspection of ex vivo Ce-lamin

Ce-lamin is encoded by a single gene, encoding for only one nuclear IF protein lamin. While classified as B-type lamin, Ce-lamin presumably serves additional A-type lamin functions. Therefore, studying Ce-lamin enables inspection of nuclear lamina assembly from a single protein. As a first insight into Ce-lamin assembly *ex vivo*, we studied spread NEs from *Xenopus* oocytes expressing Ce-lamin by field emission SEM (feSEM). Filamentous structures