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Short Communication

# The assembly of *C. elegans* lamins into macroscopic fibers

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ABSTRACT

Intermediate filament (IF) proteins are known mainly by their propensity to form viscoelastic filamentous networks within cells. In addition, IF-proteins are essential parts of various biological materials, such as horn and hagfish slime threads, which exhibit a range of mechanical properties from hard to elastic. These properties and their self-assembly nature made IF-proteins attractive building blocks for biomimetic and biological materials in diverse applications. Here we show that a type V IF-protein, the *Caenorhabditis elegans* nuclear lamin (Ce-lamin), is a promising building block for protein-based fibers. Electron cryo-tomography of vitrified sections enabled us to depict the higher ordered assembly of the Ce-lamin into macroscopic fibers through the creation of paracrystalline fibers, which are prominent in vitro structures of lamins. The lamin fibers respond to tensile force as other IF-protein-based fibers, i.e., hagfish slime threads, and possess unique mechanical properties that may potentially be used in certain applications. The self-assembly nature of lamin proteins into a filamentous structure, which is further assembled into a complex network, can be easily modulated. This knowledge may lead to a better understanding of the relationship in IF-proteins-based fibers and materials, between their hierarchical structures and their mechanical properties.



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## 1. Introduction

Biological materials execute diverse mechanical tasks in nature (Chen et al., 2008). Through evolution, their building blocks and structural design have been optimized in order to cope with a variety of environmental factors and tasks. The study of biological materials and biomimetic materials has gained interest, particularly for their potential use as alternatives for a variety of materials required by humans, especially in regenerative medicine and medical devices (Miserez et al., 2015). Among biological materials, protein-based fibers are under intense scrutiny for their exceptional mechanical properties, which combine elasticity and toughness (Gosline et al., 2002; Miserez and Guerette, 2013). Biological fibers such as spider and cocoon silks are widely used as biological materials in diverse applications (Omenetto and Kaplan, 2010). They have been elucidated in great detail in terms of structure and mechanical properties (Lintz and Scheibel, 2013). However, a complete description of their hierarchical structure and assembly pathway is still underway (van Beek et al., 2002). Another example is the native hagfish slime threads (Winegard et al., 2014), which have been shown to possess mechanical properties that are comparable to spider silk fibers (Negishi et al., 2012). The micrometer-sized slime threads are built from two intermediate filament (IF) proteins ($\alpha$ and $\gamma$) that are homologous to epidermal keratin (Koch et al., 1995). Due to the combination of impressive mechanical performance and the self-assembly nature of IF-proteins (Herrmann and Aebi, 2004), the slime threads have been proposed as biomimetic models for creating protein fibers and biomaterials with remarkable mechanics (Fudge et al., 2010). Thus, a study on vimentin, a type III cytoplasmic IF protein, showed that it can be assembled into tough fibers (Pinto et al., 2014) that, with further optimization, can reach the extraordinary mechanical properties of native hagfish threads. An improvement in fiber strength was achieved by self-assembly of recombinant vimentin into native-like 10-nm filaments, a prominent structure formed by cytoplasmic IF-proteins. It was suggested that a secondary structure transition of α-helix to β-sheets is a major contributor to strength in IF native fibers, similar to slime threads and keratin fibers (Fudge et al., 2003; Kreplak et al., 2004). In this study, we used the *Caenorhabditis elegans* lamin (Ce-lamin) as a potential building block for new macroscopic protein-based fibers (Karabinos et al., 2003).

Lamins are classified as being either A or B type (Dittmer and Misteli, 2011). Together they form a composite filaments network at the periphery of the nucleus, the nuclear lamina (Aebi et al., 1986; Shimi et al., 2008), which functions as an elastic shell (Dahl et al., 2004; Rowat et al., 2005). In vitro studies show that lamins, like all IF-proteins, consist of a central α-helical coiled-coil domain flanked by a non-α-helix 'head' and 'tail' domain. Two central α-helical domains interact to form a lamin dimer (Stuurman et al., 1998). Furthermore, lamin dimers from diverse organisms tend to form paracrystalline fibers in vitro (Heitlinger et al., 1991; Moir et al., 1991; Sasse et al., 1998). Interestingly, Ce-lamin, similar to that of mammalian lamins, forms paracrystalline fibers. In general, paracrystalline arrays have been observed in other assemblies of fibrous proteins such as collagen (Kadler et al., 1996). The variety of banding patterns that have been observed in these assemblies is mainly dependent on protein sequence, structural domains, and assembly pathways. Paracrystalline fibers assembled from lamins have been studied intensively using electron cryo-tomography (Bank et al., 2011; Ben-Harush et al., 2009; Taimen et al., 2009). These studies suggest that paracrystalline fibers are assembled in four hierarchical assembly steps (Fig. 5). First, lamin monomers coiled around each other to form a coiled-coil dimer, which is ~55 nm in length and ~2 nm thick. These dimers associate in head-to-tail fashion, with a short overlap, to form a head-to-tail polymer of dimers. Along the head-to-tail polymer axis, the distance between dimeric tails is around 48 nm (Ben-Harush et al., 2009; Stuurman et al., 1998). Next, two head-to-tail polymers interact laterally and in antiparallel fashion to form tetrameric protofilaments, ~4 nm diameter in cross-section. The distance between dimeric tails along the protofilament alternates between 21 and 27 nm, which is the result of staggered association between two head-to-tail polymers of dimers. The last step is the lateral association of protofilaments into Ce-lamin filaments or paracrystalline fibers. Therefore, the banding pattern along the paracrystalline fibers represents the distance between the C-terminus tail domains.

Ce-lamin forms stable 10-nm IF-native-like filaments, in which the proteins arrange similarly as in paracrystalline fibers. Hence, structural elements of paracrystalline fibers assemblies might keep one of the native-like structures of lamin filaments. Here, we used conditions favoring the self-assembly of Ce-lamin into paracrystalline fibers to create a network of microfibers, which then were mechanically spun into tough macroscopic fibers. Paracrystalline lamin fibers were further assembled into macroscopic fibers that resembled vimentin fibers and native hagfish threads in their response to tensile force. Therefore, simple Ce-lamin preparation might serve as a platform to better understand the relationship between hierarchical structure and mechanical properties in IF-based materials.

## 2. Material and methods

### 2.1. Ce-lamin expression and purification

Ce-lamin was expressed using BL21DE3 Rosetta plysS bacteria containing plasmid encoding *lmn-1* (NC_003279.8), and was induced using IPTG. Three hours after induction, bacteria were harvested by centrifugation. The resulting pellet was suspended in a re-suspension buffer (20 mM Tris–HCl pH=7.6, 200 mM NaCl, 1 mM EDTA, 1% v/v Triton and 1:10,000 v/v Calbiochem Protease Inhibitor Cocktail Set III). The bacterial suspension was sonicated, inclusion bodies were washed twice with the re-suspension buffer, and then incubated with 20 unit/mL Benzonaze Nuclease (Novagen, Denmark) for 30 min. Inclusion bodies were centrifuged again at 8000 g for 10 min, then dissolved in a urea buffer (20 mM Tris–HCl pH=7.6, 50 mM NaCl, 6 M urea). Lastly, the suspension was centrifuged at 16,000 g, for 1 h at 4 °C and the supernatant was kept for SDS-PAGE analysis and further use.