Journal of Structural Biology 177 (2012) 106–112

Contents lists available at SciVerse ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Structural and physiological phenotypes of disease-linked lamin mutations in *C. elegans*

Erin M. Bank [a], Kfir Ben-Harush [b], Naomi Feinstein [a], Ohad Medalia [b,c], Yosef Gruenbaum [a,*]

[a] Department of Genetics, Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904, Israel
[b] Department of Life Sciences and National Institute for Biotechnology in the Negev, Ben-Gurion University, Beer-Sheeva 84120, Israel
[c] Department of Biochemistry, University of Zurich, Zurich 8057, Switzerland

ARTICLE INFO

Article history:
Available online 7 November 2011

Keywords:
Laminopathic diseases
Nuclear lamina
EDMD
HGPS
Caenorhabditis elegans

ABSTRACT

The nuclear lamina is a major structural element of the nucleus and is predominately composed of the intermediate filament lamin proteins. Missense mutations in the human lamins A/C cause a family of laminopathic diseases, with no known mechanistic link between the position of the mutation and the resulting disease phenotypes. The *Caenorhabditis elegans* lamin (Ce-lamin) is structurally and functionally homologous to human lamins, and recent advances have allowed detailed structural analysis of Ce-lamin filaments both *in vitro* and *in vivo*. Here, we studied the effect of laminopathic mutations on Ce-lamin filament assembly *in vitro* and the corresponding physiological phenotypes in animals. We focused on three disease-linked mutations, Q159K, T164P, and L535P, which have previously been shown to affect lamin structure and nuclear localization. Mutations prevented the proper assembly of Ce-lamin into filament and/or paracrystalline arrays. Disease-like phenotypes were observed in strains expressing low levels of these mutant lamins, including decreased fertility and motility coincident with muscle lesions. In addition, the Q159K- and T164P-expressing strains showed a reduced lifespan. Thus, different disease-linked mutations in Ce-lamin exhibit major effects *in vivo* and *in vitro*. Using *C. elegans* as a model system, a comprehensive analysis of the effects of specific lamin mutations from the level of *in vitro* filament assembly to the physiology of the organism will help uncover the mechanistic differences between these different lamin mutations.

1. Introduction

The nuclear lamina is found adjacent to the nucleoplasmic face of the inner nuclear membrane (INM) and serves as both a main nuclear structural element and an interface between the nucleoplasm and the nuclear membrane (Aebi et al., 1986; Fawcett, 1966). The predominant component of the lamina is the evolutionarily conserved protein lamin, which exists as two types (A-type and B-type) in humans (Gruenbaum et al., 2003). Lamins are type-V intermediate filament (IF) proteins, characterized by their primary sequence and predicted structure (Steinert and Roop, 1988), which includes a short, unstructured amino-terminal head domain, an alpha-helical coiled-coil rod domain made of four segments of heptad repeats, and a carboxy-terminal tail domain containing a nuclear localization signal and an immunoglobulin (Ig)-fold (Ben-Harush et al., 2009; Strelkov et al., 2004).

Seminal studies by Aebi et al. revealed that lamins in the *Xenopus* germinal vesicle assemble into a network of 10-nm filaments beneath the INM (Aebi et al., 1986; Goldberg et al., 2008; Grossman et al., 2011). However, the limitation of this model is that it has not allowed the study of lamin structures in somatic cells, which remains largely unknown (Prokocimer et al., 2009). Therefore, it has been necessary to perform *in vitro* filament assembly assays, with the caveat that this system lacks lamin-binding proteins and the nuclear envelope, factors that could affect *in vivo* filament assembly (Geisler et al., 1998; Karabinos et al., 2003; Moir et al., 1991; Stuurman et al., 1998). Lamins from most species, including human lamins, assemble *in vitro* into paracrystalline arrays whose physiological relevance is not clear (Herrmann and Foisner, 2003; Klapper et al., 1997; Melcer et al., 2007; Moir et al., 1991; Stuurman et al., 1998; Taimen et al., 2009). Importantly, *Caenorhabditis elegans* lamin (Ce-lamin) assembles *in vitro* into either paracrystalline arrays or 10 nm IF-like filaments depending on the specific assembly conditions (Foeger et al., 2006; Karabinos et al., 2003). Interestingly, both structures have a similar basic arrangement of

Abbreviations: dsRNA, double stranded RNA; EDMD, Emery–Dreifuss muscular dystrophy; ET, electron tomography; IF, intermediate filament; TEM, transmission electron microscopy.

* Corresponding author.
E-mail address: gru@vms.huji.ac.il (Y. Gruenbaum).

1047-8477/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2011.10.009

lamin protofilaments: two antiparallel head-to-tail polymers of lamin dimers come together to form tetrameric protofilaments consisting of four lamin polypeptides (Ben-Harush et al., 2009). This has made *C. elegans* a powerful model organism to study lamin functions from the level of *in vitro* filament assembly to the physiology of the animal. Ce-lamin is encoded by a single gene (*lmn-1*) that is functionally similar to both human A- and B-type lamin proteins (Liu et al., 2000; Melcer et al., 2007; Riemer et al., 1993). Because of this conservation, mutations in Ce-lamin residues homologous to *LMNA* residues mutated in laminopathic diseases can be studied *in vitro* and *in vivo*.

Laminopathies are a class of human heritable diseases predominately caused by mutations in the human A-type lamin gene (*LMNA*), of which >400 mutations and >14 diseases have been identified, all of which vary greatly in severity and tissue specificity (Broers et al., 2006; Mattout et al., 2006; Worman and Bonne, 2007). Among the autosomal-dominant laminopathies are the muscle diseases Emery–Dreifuss muscular dystrophy (EDMD), *LMNA*-related congenital muscular dystrophy (L-CMD), limb-girdle muscular dystrophy, and dilated cardiomyopathy; the aging and skin disorder restrictive dermopathy; the neuropathic Charcot-Marie-Tooth disorder; the lipodystrophies mandibuloacral dysplasia and Dunnigan-type familial partial lipodystrophy; and the systemic premature aging syndromes Hutchison–Gilford progeria syndrome (HGPS) and atypical Werner syndrome (Quijano-Roy et al., 2008; Vlcek and Foisner, 2007). To date, no correlation has been established between the position of the lamin mutation and the resulting disease symptoms (Worman and Bonne, 2007).

Previous studies described the varying effects of lamin mutations on the *in vitro* assembly of paracrystalline fibers and filaments (Ben-Harush et al., 2009; Wiesel et al., 2008). Expression of these mutant lamins in *C. elegans* revealed that each had a different pattern of mislocalization within the nucleus and alterations in protein dynamics (Wiesel et al., 2008). These preliminary data already reveal the varying pathologies caused by lamin mutations in human. In this paper, we focus on three such mutations: Q159K (E145K in *LMNA*), which is associated with HGPS, and T164P (T150P in *LMNA*) and L535P (L530P in *LMNA*), which are associated with EDMD. We have previously shown that all three mutations affect *in vitro* filament assembly, as seen by negative staining electron microscopy, and localization within the nucleus of mutant animals (Wiesel et al., 2008). Here, we use high-resolution cryo-electron tomography (cryo-ET) to show that mutations prevent the proper assembly of Ce-lamin into IF-like 10-nm filaments and paracrystalline arrays. Disease-like phenotypes were observed in strains expressing an exogenous copy of these mutant lamins: all three strains had decreased fertility and motility coincident with muscle lesions, and the Q159K and T164P strains also had reduced lifespan. Together, our data establish *C. elegans* as a model system in which to study laminopathic diseases. By understanding the effect of specific lamin mutations from the level of *in vitro* filament assembly to the physiology of the organism, we hope to establish a clearer mechanistic picture of how laminopathic diseases occur.

2. Materials and methods

2.1. Constructs and bacterial expression of Ce-lamin

Ce-lamin cDNA in pET24d (Foeger et al., 2006) was mutagenized by site-directed mutagenesis as described (Wiesel et al., 2008). Plasmids were used to transform *Escherichia coli* BL21(DE3)-(codon plus-RIL), expressed by IPTG induction, and the proteins purified with Ni–NTA beads as described (Foeger et al., 2006).

Worm strains were previously generated by our laboratory (Wiesel et al., 2008). Briefly, wildtype and mutant *lmn-1* were cloned into the pAD010 vector, which includes the *baf-1* promoter (Margalit et al., 2007), the *unc-119* gene, and a *gfp* gene with *C. elegans* introns. Constructs were introduced into worms by microparticle bombardment as described (Praitis et al., 2001). To remove background mutations, the resulting strains were outcrossed to wildtype (N2) worms three times. Each of the mutant strains expresses 10–20% GFP-tagged Ce-lamin levels as compared to the endogenous Ce-lamin levels (Bank et al., 2011; Wiesel et al., 2008).

2.2. Assembly of Ce-lamin filaments

Bacterially expressed and purified *C. elegans* lamin (0.1–0.2 mg/ml) in urea-containing buffer was dialyzed at room temperature against buffer containing 2 mM Tris–HCl, pH 9.0, 1 mM DTT for 4 h, followed by dialysis for 16 h against a buffer containing 15 mM Tris–HCl, pH 7.4, 1 mM DTT, as described (Foeger et al., 2006).

2.3. Cryo-electron tomography

Lamin filaments and paracrystalline fibers were prepared and images processed as described (Ben-Harush et al., 2009). Briefly, lamin structures were applied onto a glow-discharged 200-mesh carbon-coated copper grid (Quantifoil, Jenna, Germany) to which colloidal gold was added, followed by vitrification (Dubochet et al., 1988). Data were collected under cryo conditions using a 300 kV FEI Polara transmission electron microscope equipped with a field-emission gun, and a Gatan postcolumn GIF 2002 energy filter. Tilt series were collected over an angular range of 60° to −60°, with a 2° increment, with a defocus value of −10 μm. The resulting pixel size was 4.25 Å for lamin filaments and 5.25 Å for paracrystalline fibers, at the specimen level. Thus, the theoretical resolution limit, as set by the contrast transfer function, was 38–44 Å. The projection images (2048 × 2048) were aligned to a common frame using fiducial gold markers, and reconstructed by weighted back-projection, as implemented by the TOM toolbox software package (Nickell et al., 2005).

2.4. Fertility experiments

Healthy L4 larval stage worms with GFP expression were picked individually to plates and transferred to a fresh plate each subsequent day during their gravid lifespan. Progeny from each day were scored after 24 h to ensure they had time to hatch. Four independent experiments were performed each containing at least 10 worms of each strains.

2.5. Motility experiments

Healthy L4 animals with GFP expression were transferred to an NGM plate without food. After a few seconds, the number of head turns in one minute was scored. Six independent experiments were performed each containing at least 10 worms of each strain.

2.6. Aging experiments

Healthy L4 animals with GFP expression were transferred to an NGM plate containing fluorodeoxyuridine (FuDR) to prevent new progeny from developing (Mitchell et al., 1979). Worms were kept at 23 °C for the duration of the experiment. Worms were moved to a new plate every 2 days to ensure ample supply of food; dead worms were removed and scored at this time. Each experiment was performed in triplicate with $n = 60$ (30 worms/plate).