The Single Nuclear Lamin of Caenorhabditis elegans Forms In Vitro Stable Intermediate Filaments and Paracrystals with a Reduced Axial Periodicity

Anton Karabinos1, Jürgen Schünemann1, Michael Meyer1, Ueli Aebi2 and Klaus Weber1*

1Max Planck Institute for Biophysical Chemistry
Department of Biochemistry
Am Fassberg 11, 37077 Göttingen, Germany
2M. E. Müller-Institute for Microscopy at the Biozentrum
University of Basel
Klingelbergstrasse 70, CH-4056 Basel, Switzerland

The lamins of the tunicate Ciona intestinalis and the nematode Caenorhabditis elegans show unusual sequence features when compared to the more than 35 metazoan lamin sequences currently known. We therefore analyzed the in vitro assembly of these two lamins by electron microscopy using chicken lamin B2 as a control. While lamin dimers usually appear as a rod carrying two globules at one end, these globules are absent from Ciona lamin, which lacks the central 105-residue region of the tail domain. The deletion of 14 residues or two heptads from the coiled coil rod domain of the single C. elegans lamin results in a 1.5-nm shortening of the dimer rod. Similarly, the paracrystals assembled from the C. elegans lamin exhibit a 3.1-nm reduction of the true axial repeat compared to that of chicken lamin B2 paracrystals. We speculate that the banding pattern in the C. elegans lamin paracrystals arises from a relative stagger between dimers and/or a positioning of the globular tail domain relative to the central rod that is distinct from that observed in chicken lamin B2 paracrystals. Here we show that a nuclear lamin can assemble in vitro into 10-nm intermediate filaments (IFs). C. elegans lamin in low ionic strength Tris-buffers at a pH of 7.2–7.4 provides a stable population of lamin IFs. Some implications of this filament formation are discussed.

Keywords: Ciona intestinalis; Caenorhabditis elegans; intermediate filament; lamin; paracrystals

Abbreviation used: IF, intermediate filament.
E-mail address of the corresponding author: akarabi1@gwdg.de

0022-2836/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved
Nuclear lamins seem restricted to metazoa, as the completed genomes of the yeast Saccharomyces cerevisiae and the plant Arabidopsis thaliana lack obvious orthologs. Some 35 lamin sequences are currently known. They range from the simplest animals such as the cnidarians (Hydra and Taelia) to various other invertebrates (Caenorhabditis elegans, Drosophila, Priapulus, several echinoderms, two urochordates and the cephalochordate Amphioxus), and to a large number of vertebrates. Alignment of these sequences defined the variable and conserved domains and identified a rare lamin species that deviates from the general theme (Figure 1). Of variable length are the relatively small (typically 30–60 residues) head domain, the region between the C-terminal end of the rod domain and the nuclear localization signal, as well as the region between this signal and the well conserved central part of the tail, the so called 105-residue region whose atomic structure has recently been solved as an Ig-like domain for human lamin A both by X-ray crystallography and by NMR spectroscopy. Length variability continues in the rest of the tail domain. This domain also includes the additional exon (about 95 residues) inserted in the larger A-type lamins that are restricted to the vertebrates. Nearly all lamin sequences end with the motif CaaX. In vivo this motif is processed to a cysteine residue that, in turn, is carboxyl-methylated and carries an isoprenyl derivative in thio-ether linkage. In contrast to the two end domains the central rod domain is nearly canonically conserved in length. Even in cnidarian lamins there is only minimal variability. The linker L2 between coils 1b and 2a is increased by one and three residues in Hydra and Taelia lamin, respectively. A much larger change occurs only in the lamin rod of the nematode C. elegans, which lacks 14 residues or two heptads early in coil 2b. The C. elegans lamin arises from the only lamin gene in the genome, and Northern analysis indicates a single mRNA species. Yet another length variability arises in the vertebrate A lamins (sequences not shown). They have an additional exon inserted in the tail domain (approximate position marked by the arrow pointing upwards). It is inserted past the acidic cluster found in all vertebrate lamins. The unique features of two lamins are underlined: the C. elegans lamin harbors a 14-residue deletion within the rod domain, i.e. early in coil 2b, and the Ciona lamin L1 lacks the Ig-like 105-residue region within the tail domain (see the text).
was as above. Scale bar represents 50 nm.

50 mM NaCl, 1 mM DTT, 50 mM Tris–HCl (pH 8.0) at room temperature. Glycerol spraying/rotary metal shadowing

Figure 2. Purification of recombinant lamins (a), dimer formation ((b), (c) and (d)), and head-to-tail formation of dimers (e). Purification of recombinant lamins (a). The coding sequence of *C. elegans* lamin\(^1\) was amplified by PCR from an embryonic Uni-ZAP cDNA library (Stratagene, Heidelberg, Germany) and ligated into the expression vector pET22 (Novagen, Madison, WI, USA). *E. coli* BL21 (DE3) Lys S cells containing the expression plasmid were grown at 37 °C in 200 ml SOB medium\(^2\) with 50 µg/ml ampicillin. Expression was induced with 1 mM IPTG in late exponential phase. After three to four hours the cells were pelleted, washed and resuspended in guanidinium lysis buffer (6 M guanidine hydrochloride, 20 mM sodium phosphate, 500 mM NaCl, pH 7.8) containing a protease inhibitor cocktail (Roche, Mannheim, Germany). After incubation at room temperature for ten minutes, sonication and centrifugation at 10,000 × g the supernatant was applied onto a ProBond\(^7\) column equilibrated with denaturing buffer (8 M urea, 20 mM sodium phosphate, 500 mM NaCl, pH 7.8). The eluate obtained by the same buffer at pH 6 that was highly enriched in recombinant *C. elegans* lamin, was applied onto a fast desalting 26/10 column (Amersham Pharmacia, Uppsala, Sweden) that was equilibrated in 8.5 M urea containing 10 mM Na₂HPO₄, 1 mM 2-mercaptoethanol (pH 6.6). The protein fraction obtained was subjected to ion exchange chromatography on Mono S equilibrated in the same buffer. Fractions containing pure lamin were pooled, passed through a fast desalting column in 8.5 M urea containing 10 mM Tris–HCl, 1 mM 2-mercaptoethanol (pH 8) and subjected to ion exchange chromatography on Mono Q equilibrated in the same buffer. Fractions containing pure *C. elegans* lamin were identified by SDS-PAGE. The coding sequence of chicken lamin B2 cDNA was amplified by PCR from the vector pAR3038\(^8\) and subcloned into the bacterial expression vector pET23 vector (Novagen). After expression in *E. coli* and ligated into the pET23 vector (Novagen). After expression in *E. coli*, inclusion bodies highly enriched in recombinant *Ciona* lamin, was solubilized in 8.5 M urea. Further purification of *Ciona* lamin by ion exchange chromatography on Mono S and Mono Q provided a pure preparation as assayed by SDS-PAGE. All purified lamins were stored in aliquots in 8.5 M urea Tris–HCl buffer at −20 °C. Lamin dimer formation ((b)–(d)). Aliquots of *C. elegans*, chicken and *Ciona* lamins in urea buffer were dialyzed for two hours against 150 mM NaCl, 1 mM EGTA in 25 mM Tris–HCl (pH 8.5) at protein concentrations of 0.4 mg/ml, 0.5 mg/ml and 0.25 mg/ml, respectively. Dialysis was at room temperature using dialysis tubing with a molecular weight cut-off of 35,000 (Millipore, Eschborn, Germany). All buffers were 1 mM in DTT and were made in Milli-Q water (Milli-Q standard system in organex configuration; Millipore). For glycerol spraying/rotary metal shadowing 30 m aliquots of dialyzed protein were mixed with glycerol to a final concentration of 30% and sprayed onto freshly cleaved mica. The mica was placed on the rotary table of a Balzers apparatus (BAE 120T; Balzers Pfeiffer GmbH, Asslar, Germany) and dried at room temperature in vacuo. Dried samples were rotary shadowed with platinum/carbon at an elevation angle of 3.5° and coated with a carbon film. Specimens were observed using a Philips CM12 transmission electron microscope (Philips, Eindhoven, The Netherlands) operated at 80 kV. Electron micrographs were digitised with an Agfa Duoscan T2500 scanner at a resolution of 1200 dpi. Digitised micrographs were processed using Adobe Photoshop software. Lamin dimers of *C. elegans* (b), chicken B2 (c) and *Ciona* (d). Note the absence of the two globules at one end of the rod in the case of *Ciona* lamin dimers. Longitudinal head-to-tail polymers of *C. elegans* lamin dimers (e). Lamin in urea buffer was dialyzed for one hour against 50 mM NaCl, 1 mM DTT, 50 mM Tris–HCl (pH 8.0) at room temperature. Glycerol spraying/rotary metal shadowing was as above. Scale bar represents 50 nm.

Using recombinant proteins purified from *Escherichia coli* we have analyzed the in vitro assembly of the two unusual lamins by electron microscopy. For comparison, chicken lamin B2 served as a representative of normal lamins. The three lamins were expressed in *E. coli* and the proteins were purified in urea buffers. Figure 2(a) documents the purity of the preparations. Lamins
in 8 M urea at 0.25–0.5 mg/ml were briefly dialyzed against 150 mM NaCl, 1 mM DTT, 25 mM Tris–HCl (pH 8.5), and processed for glycerol spraying/rotary metal shadowing. Figure 2(b) shows that dimers of *C. elegans* lamin have the same ultrastructural appearance as chicken lamin B2 dimers (Figure 2(c)), which were extensively studied in the past.\(^6,8\) Both molecules display a rod with two distinct globules situated at one end. Evidently, these globules represent a distinct part of the C-terminal tail domain of these lamins.\(^2,6,15\) In fact, the Ig-like 105-residue region\(^11,12\) of human lamin A (i.e. residues 434–551), when expressed in *E. coli*, appears after glycerol spraying/rotary metal shadowing as globules indistinguishable in size and shape from those depicted in the intact lamin dimers (K. Bechert, our unpublished results). In line with this view are the images of the *Ciona* lamin dimer, which misses the 105-residue region in its tail domain (Figure 1) and hence lacks the two pronounced globules at one end of its rod domain (Figure 2(d)). Measurements on 100 molecules show that the rod domain of chicken lamin (54.2(±1.2) nm) is slightly longer than the rod domain of *C. elegans* lamin (52.7(±1.4) nm). This 1.5-nm length difference is in the range predicted for a 14-residue deletion (Figure 1) in coiled-coil conformation (calculated value 2 nm). As described for rat, chicken and *Drosophila* lamin dimers\(^1,7,8\) the *C. elegans* lamin dimers also formed pronounced head-to-tail polymers when dialyzed for one hour against 50 mM NaCl, 1 mM DTT, 20 mM CaCl\(_2\). All buffers were 1 mM in DTT. Negative staining was with 2% (w/v) uranyl acetate. Note the different banding patterns of the *C. elegans* and chicken B2 lamin paracrystals (for details see the text). Scale bar represents 200 nm.

---

**Figure 3.** Lamin paracrystal formation. (a) *C. elegans* lamin at 0.4 mg/ml in urea buffer was dialyzed for one hour against 15 mM Tris–HCl (pH 7.4) followed by a two hour dialysis against 25 mM Tris–HCl (pH 9.0), 20 mM CaCl\(_2\). (b) Chicken lamin B2 at 0.2 mg/ml was first dialyzed against 15 mM Tris–HCl (pH 7.4) for 20 hours and then for five hours against 25 mM Tris–HCl (pH 9.0), 25 mM CaCl\(_2\). (c) *Ciona* lamin at 0.2 mg/ml was dialyzed against 20 mM Tris–HCl (pH 8.0), 50 mM NaCl for one hour and then for four hours against 20 mM Tris–HCl (pH 7.2), 20 mM CaCl\(_2\). All buffers were 1 mM in DTT. Negative staining was with 2% (w/v) uranyl acetate. Note the different banding patterns of the *C. elegans* and chicken B2 lamin paracrystals (for details see the text). Scale bar represents 200 nm.
light bands and amounts to 47.8(\pm 0.4) \text{ nm} (100 measurements). The 3.1-nm shorter axial repeat in the case of \textit{C. elegans} (Figure 3(a)) is most likely due to the deletion of two heptads from the coiled-coil rod domain of \textit{C. elegans} lamin (Figure 1) and is in line with the slightly reduced length of the lamin dimer molecules (Figure 2), i.e. 52.7 nm \textit{versus} 54.2 nm (see above). Similarly, the different spacings of the transverse bands within the axial repeat of \textit{C. elegans} \textit{versus} chicken B2 lamin paracrystals may, at least in part, arise too from the missing two heptads in coil 2 of \textit{C. elegans} lamin, as this yields a different stagger between antiparallel dimers that interact \textit{via} their coil 2 (i.e. the A22 dimer–dimer interaction\textsuperscript{17}). In addition, and possibly as a consequence of its shorter coil 2, the axial positioning of the globular tail domain in the \textit{C. elegans} lamin paracrystals may be distinct from that in chicken lamin B2 paracrystals.

The paracrystals formed by \textit{Ciona} lamin appeared much poorer in quality than those obtained from the other two lamins (Figure 3(c); compare with Figure 3(a) and (b)). Nevertheless, in many regions a distinct banding pattern could be observed and resembled that of chicken lamin B2 both in terms of appearance and axial repeat length. Since previous studies with truncated lamins lacking the entire tail domain have documented that they retain the ability to form paracrystals\textsuperscript{6,7,15,16,18–20} we have not tried to improve the quality of the paracrystals formed by the \textit{Ciona} lamin that lacks the central part (i.e. the 105-residue globule) of the tail domain (see Figure 1).

Previous studies on the \textit{in vitro} assembly of chicken, human, rat and \textit{Drosophila} lamins\textsuperscript{16–8,15} emphasized that intermediate filament-like structures (i.e. exhibiting a \textit{\approx} 10-nm average diameter)
are only transiently observed and absent at steady state where thick filament bundles, aggregates and paracrystalline arrays predominate. To see whether assembly conditions can be established in which C. elegans lamin forms stable IFs, we employed various low ionic strength buffers that are known to yield 10-nm filaments with invertebrate cytoplasmic IF proteins 21–23 and with mammalian keratins. 24–27 The best results were achieved when C. elegans lamin (at 0.1 mg/ml) was dialyzed against 15 mM Tris–HCl (pH 7.4). Figure 4(a) and (b) reveals IFs with an average diameter of 10.2(± 0.7) nm (Figure 4(b), inset) that were obtained under these conditions in two separate experiments. Filament bundling was only rarely observed and no paracrystals were seen.

Although these low ionic strength buffers also yielded filaments with chicken B2 and Ciona lamin (Figure 4(c) and (d)), the resulting filament populations appear much more polymorphic. Chicken lamin B2, for example, formed filaments with an average diameter of 9.6(± 2.7) nm, i.e. very close to that of bona fide IFs. While these filaments exhibited a strong tendency to form tangles (Figure 4(c)), no paracrystals were detected under these assembly conditions. The Ciona filaments, in contrast, yielded a markedly smaller average diameter, i.e. 5.4(± 1.1) nm (Figure 4(d)), than is common for bona fide IFs.

Here we have documented that the single nuclear lamin of the nematode C. elegans, 14 assembles in low ionic strength Tris–HCl buffer into stable 10-nm filaments that exhibit a normal IF morphology (see Figure 4(b)). This result is in line with the finding that the primary sequences of nuclear lamins and cytoplasmic IF proteins are related 28, 29 and that at least in Xenopus oocytes the nuclear lamina is built as a near tetragonal lattice of 10-nm filaments. 1 Nevertheless previous studies using different buffer conditions found that lamina assembly notoriously leads to thick bundles, tangles and paracrystalline arrays and that 10-nm-like filaments are practically absent at steady state (for references see above). However the organisation of the nuclear lamina in somatic metazoan cell types is not known. Thus it remains to be seen whether such laminae are also built from a sizable amount of bona fide 10-nm lamin filaments, or whether they are formed by thinner filaments such as those that form during early in vitro assembly stages by head-to-tail polymerization of lamin dimers or several such filaments after lateral association. 8

Acknowledgements

We thank Uwe Plessmann for protein sequencing. We thank Dr Erich Nigg, Munich, for providing the chicken B2 cDNA and Dr Georg Krohne, Würzburg, for a gift of recombinant Drosophila lamin Dmo. This work was supported in part by a grant from the German-Israeli Foundation to K.W., by a grant from the Swiss National Science Foundation to U.A., and by the Maurice E. Müller Foundation of Switzerland.

References


(Received 26 July 2002; received in revised form 25 October 2002; accepted 31 October 2002)