The Last Twenty Residues in the Head Domain of Mouse Lamin A Contain Important Structural Elements for Formation of Head-to-Tail Polymers in Vitro

Kazuhiro ISOBE,1 Rumi GOHARA,1 Toshihisa UEDA,2 Yozo TAKASAKI,1 and Shoji ANDO1,†

1Division of Biopolymer Research, Department of Biomolecular Sciences, Faculty of Medicine, Saga University, Nabeshima, Saga 849-8501, Japan
2Department of Applied Biochemistry and Food Science, Faculty of Agriculture, Saga University, Honjo, Saga 840-8502, Japan

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Nuclear lamins are a type of intermediate filament (IF) proteins. They have a characteristic tripartite domain structure with an $z$-helical rod domain flanked by non-$z$-helical N-terminal head and C-terminal tail domains. While the head domain has been shown to be important for the formation of head-to-tail polymers that are critical assembly intermediates for lamin IFs, essential structural elements in this domain have remained obscure. As a first step to remedy this, a series of mouse lamin A mutants in which the head domain (30 amino acid residues) was deleted stepwise from the N-terminus at intervals of 10 residues were bacterially expressed. The assembly properties in vitro of the purified recombinant proteins were explored by electron microscopy. We observed that while a lamin A mutant lacking N-terminal 10 residues formed head-to-tail polymers, a mutant lacking N-terminal 20 residues or the whole head domain (30 residues) showed significantly decreased potency to form head-to-tail polymers. These results suggest that the last 20 residues (from Arg-11 to Gln-30) of the head domain of mouse lamin A contain essential structures for the formation of head-to-tail polymers. The last 20 residues of the head domain include several conserved residues between A- and B-type lamins and also the phosphorylation site for cdc2 kinase, which affects lamin IF organization in vivo and in vitro. Our results provide clues to the molecular mechanism by which the head domain plays a crucial role in lamin polymerization.

Key words: cytoskeleton; head-to-tail polymer; intermediate filaments; lamin A

The nuclear lamina is a filamentous meshwork lining the nucleoplasmic surface of the nuclear envelope. It serves as a structural framework of the nucleus in protecting it from physical damage, and also as an anchoring site for nuclear pore complexes.1) The major structural proteins of the nuclear lamina are lamins, type-V intermediate filament (IF) proteins.1–3) Nuclear lamins are further classified as either A-type or B-type depending on their primary sequences and tissue-specific expression patterns. B-type lamins are constitutively expressed in all embryonic and somatic tissues. A-type lamins, lamin A and lamin C, are alternatively spliced products of the same gene, and are expressed exclusively in differentiated cells and tissues. Like other cytoplasmic IF proteins, nuclear lamins possess three distinct structural domains: the N-terminal head domain, the central rod domain, and the C-terminal tail domain.2–8) The central rod domain of lamins is about 350 amino acids in length and can be divided into four $z$-helical subdomains (coils 1A, 1B, 2A, and 2B), each of which is organized around the heptad repeats6) and separated by linkers termed L1, L12, and L2. The N-terminal head domain of nuclear lamins is typically 30–60 amino acids in length, whereas the C-terminal tail domain is 210–300 amino acids long. In lamins, but not in cytoplasmic IF proteins, the tail domain contains a nuclear localization signal.2,3) The tail domain of lamin A and the B-type lamins contains a CaaX box (C, cysteine; a, aliphatic amino acid; X, any amino acid) at the C-terminus that serves as a site for modification by isoprenylation and methylation.2,3) Lamin C, a splicing variant of A-type lamin, lacks the final 82 amino acids, including the CaaX box. A- and B-type lamins differ in that the CaaX motif in lamin A is removed by proteolytic cleavage after nuclear import.
In vitro assembly of nuclear lamins from human, chicken, rat, Xenopus, and Drosophila has been studied.1,9–14 At the first level of structural organization, two lamin polypeptides associate laterally into a parallel, unstaggered coiled-coil dimer via their α-helical rod domains. Under electron microscopy of glycerol-sprayed/rotary metal-shadowed specimens, lamin dimer appears as an approximately 50-nm long rod with two globules at one end.1,9–14 These globules represent the C-terminal tail domain of the lamins. The central rod domain is sufficient for lamin dimer formation. At the second level of structural organization, lamin dimers associate longitudinally into polar head-to-tail polymers. This head-to-tail association involves an “overlap” of the highly conserved N-terminal segment of coil 1A and the highly conserved C-terminal segment of coil 2B by 2–4 nm, which are assumed to be formed through lateral association of the head-to-tail polymers, are observed only transiently during in vitro assembly of lamins, and are practically absent in the steady state.1,10 although in situ lamins clearly do assemble into long, uniform IFs in nuclear lamina. Hallmarks within primary structures of nuclear lamins that lead to observed assembly properties different from those of cytoplasmic IF proteins15–17 are not fully understood.

The head domain of lamins is indispensable for formation of head-to-tail polymers in vitro11,14 and also for lamin IF formation in vivo.18–20 Phosphorylation/ dephosphorylation of the head domain induces disassembly/assembly of lamin IF networks in vitro21–23 and head-to-tail polymers in vitro.23,24 However, important structural elements of the head domain for lamin polymerization are unsolved. While arginine residues in the head domain of cytoplasmic IF proteins have been found to be involved in IF formation in vitro and in vivo,25–31 the significance of arginine residues in the head domain of nuclear lamins remains to be characterized. Other hydrophilic and hydrophobic residues might be also important in lamin assembly.4,6,27 As a first step to localize essential structural elements in the head domain of lamins, we engineered stepwise deletions of the head domain (30 amino acid residues) of mouse lamin A from the N-terminus at intervals of 10 amino acid residues. The assembly properties in vitro of the purified recombinant proteins were analyzed by electron microscopy. The results obtained suggest that the last 20 residues of the head domain of mouse lamin A contain essential structures for the head-to-tail association of dimers, a first critical step in lamin polymerization.

Materials and Methods

Plasmid construction. Full-length mouse lamin A cDNA (2 kb)p12,33 flanked by 5′ NdeI and 3′ EcoRI restriction sites was constructed by PCR amplification using a cDNA library from mouse liver as template and primers LamA-5′-NdeI (5′-AACATATGAGACCCCCGTCACACGGG-3′) and LamA-3′-EcoRI (5′-GTGAAATTCTTCACTATGCTGCTGTTCTG-3′), cloned into pT7blue plasmid (Novagene, Madison, WI), and confirmed by DNA sequencing. This construct (pT7-LamA) was digested with NdeI and EcoRI, and the resulting DNA fragments were isolated and then subcloned into pET24c plasmid (Novagene) which had been digested with NdeI and EcoRI. The identity of this construct (pET24c-LamA) was confirmed by restriction analysis and by expression in E. coli strain BL21(DE3)pLysS (Novagene).

DNA encoding the “head domain-deletion” mutants LamA(ΔN10), LamA(ΔN20), and LamA(ΔHD) were amplified by the PCR using pET24c-LamA as a template and primers LamA-3′-EcoRI and LamA(ΔN10)-5′-NdeI (5′-GCCATATGCGCAGTGGGGCGCAG-3′), LamA(ΔN20)-5′-NdeI (5′-CTCATATGCTGTCGGCCACTCGG-3′), and 1a(LamA)-5′-NdeI (5′-GCCATATGAGACCGGACCTCGGACAG-3′) respectively. Primers LamA(ΔN10)-5′-NdeI, LamA(ΔN20)-5′-NdeI, and 1a-LamA)-5′-NdeI introduced an initiation codon and NdeI site before Arg-11, Leu-21, and Glu-31 (the first residue of coil 1A) respectively. The PCR products were cloned into pT7blue plasmid, confirmed by DNA sequencing, and then subcloned into pET28b plasmid which had been digested with NdeI and EcoRI. The identity of the constructs was confirmed by restriction analysis and by expression in BL21(DE3)pLysS.

Expression and purification of mouse lamin A and its mutants. Full-length mouse lamin A (77.7 kDa) was expressed in BL21(DE3)pLysS using pET24c vector and isopropyl-β-D-thiogalactopyranoside (1 mM) induction. To get sufficient amounts of mutants LamA(ΔN10) (76.6 kDa), LamA(ΔN20) (75.6 kDa), LamA(ΔHD) (74.5 kDa), they were expressed using pET28b vector, expressed in BL21(DE3) strain BL21(DE3)pLysS (Novagene).

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100,000 × g for 60 min at 4°C. The supernatant was loaded onto a Q-Sepharose column (Amersham Biosciences, Piscataway, NJ) that was eluted with a linear 0–0.6 M NaCl gradient in buffer I (10 mM Tris–HCl, pH 9.0, 1 mM EDTA, 10 mM β-mercaptoethanol, 0.5 mM PMSF, 6 M urea). The fractions containing the objective protein were pooled, dialyzed against buffer II (20 mM MES–NaOH, pH 6.0, 1 mM EDTA, 10 mM β-mercaptoethanol, 0.5 mM PMSF, 6 M urea), and then loaded onto a SP-Sepharose column (Amersham) that was eluted with a linear 0–0.6 M NaCl gradient in buffer II. The fractions containing the objective protein were pooled, dialyzed against buffer III (10 mM Tris–HCl, pH 9.0, 2 mM EDTA, 10 mM β-mercaptoethanol, 250 mM NaCl, 8 M urea), and stored at −20°C until use. Use of Ni²⁺-immobilized resin (Novagen) for purification of His-tagged LamA(C1N10), LamA(C1N20), and LamA(CHD) was abandoned, since the objective proteins precipitated after elution from the resin with 20 mM Tris–HCl, pH 8.0, 100–200 mM imidazole, 500 mM NaCl, 6 M urea. After purification, the concentration of each protein was determined spectrophotometrically, using bovine serum albumin as the standard.

In vitro assembly of mouse lamin A and its mutants. For dimer formation, each protein (0.25 mg/ml) in 0.5 ml of buffer III was dialyzed against 500 ml of dimerization buffer (25 mM Tris–HCl, pH 8.5, 1 mM EDTA, 1 mM dithiothreitol, 150 mM NaCl) at room temperature for 2–3 h. For head-to-tail polymerization of dimers, each protein (0.25 mg/ml) in 0.5 ml of buffer III was dialyzed against 500 ml of dimer-elongation buffer (50 mM Tris–HCl, pH 8.0, 1 mM dithiothreitol, 50 mM NaCl) at room temperature for 3–6 h. Paracrystal formation was induced by dialysis of each protein (0.25 mg/ml) in 0.5 ml of buffer III against 500 ml of paracrystal forming buffer (25 mM MES–NaOH, pH 6.5, 1 mM EDTA, 1 mM dithiothreitol, 150 mM NaCl) at room temperature for 2–6 h.

**Electron microscopy.** For glycerol spraying/low-angle rotary metal shadowing, an aliquot (70 µl) of each sample was mixed with glycerol to a final concentration of 30% and sprayed onto freshly cleaved mica. The mica piece was placed on the rotary table and dried in vacuo at room temperature for 10 min. Then the sample was rotary shadowed with platinum/carbon at an elevation angle of 5°. For negative staining, an aliquot (30 µl) of each sample was adsorbed for 3 min to a glow-discharged carbon-coated collodion film on a copper grid. Then the grid was exposed to 2% uranyl acetate for 30 s. After removal of excess liquid with filter paper, the sample was permitted to air dry. Specimens were examined using a JEM-1210 transmission electron microscope (JEOL, Tokyo) operated at an accelerating voltage of 80 kV.

**Results**

**Bacterial expression of full-length mouse lamin A and deletion mutants**

The structures of full-length mouse lamin A and deletion mutants expressed in this study are shown schematically in Fig. 1. As a first step to localize essential structural elements in the head domain of mouse lamin A, we constructed a series of mutants, LamA(ΔN10), LamA(ΔN20), and LamA(CHD), in which the head domain (30 amino acid residues) was deleted stepwise from the N-terminus at intervals of 10 amino acid residues. The interval was fixed, since the absolute size of the deletion, besides the position of the deletion, might have affected lamin polymerization in vitro. Use of Ni²⁺-immobilized resin (Novagen) for purification of His-tagged LamA(ΔN10), LamA(ΔN20), and LamA(CHD) was abandoned, since the objective proteins precipitated after elution from the resin with 20 mM Tris–HCl, pH 8.0, 100–200 mM imidazole, 500 mM NaCl, 6 M urea. After purification, the concentration of each protein was determined spectrophotometrically, using bovine serum albumin as the standard.

In vitro assembly of mouse lamin A and its mutants. For dimer formation, each protein (0.25 mg/ml) in 0.5 ml of buffer III was dialyzed against 500 ml of dimerization buffer (25 mM Tris–HCl, pH 8.5, 1 mM EDTA, 1 mM dithiothreitol, 150 mM NaCl) at room temperature for 2–3 h. For head-to-tail polymerization of dimers, each protein (0.25 mg/ml) in 0.5 ml of buffer III was dialyzed against 500 ml of dimer-elongation buffer (50 mM Tris–HCl, pH 8.0, 1 mM dithiothreitol, 50 mM NaCl) at room temperature for 3–6 h. Paracrystal formation was induced by dialysis of each protein (0.25 mg/ml) in 0.5 ml of buffer III against 500 ml of paracrystal forming buffer (25 mM MES–NaOH, pH 6.5, 1 mM EDTA, 1 mM dithiothreitol, 150 mM NaCl) at room temperature for 2–6 h.

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Ser-18), in addition to Thr-19 and Pro-20, which are highly conserved in both A- and B-type lamins. The C-terminal 10 residues (from Leu-21 to Gln-30) are highly conserved in both A- and B-type lamins, and include two arginine residues (Arg-25 and Arg-28) and the phosphorylation site for cdc2 kinase, which induces disassembly of lamin IFs and the head-to-tail polymers.21–24 Thus the five arginine residues in the head domain of mouse lamin A are shared out among the three segments composed of 10 amino acid residues.

While full-length lamin A was expressed in sufficient amounts in BL21(DE3)pLysS cells by pET24c vector and isopropyl-β-D-thiogalactopyranoside induction, the deletion mutants were not expressed sufficiently. To improve bacterial expression of these mutants, we used pET28b as an alternative expression vector, which resulted in the addition of the His-tag sequence (MGSSHHHHHHSSGLVPRGSH, 2.1 kDa) to their N-terminus. All of the bacterially expressed proteins were obtained in inclusion bodies and were purified by conventional ion-exchange column chromatography in the presence of 6M urea, as described in "Materials and Methods." Removal of the His-tag sequence from the N-terminus of the lamin mutants by thrombin was abandoned, because unwanted proteolysis, besides proteolysis at the designed site between the His-tag sequence and the lamin A sequence, was observed under the conditions examined. Since it has been reported that the addition of the hemiglutinin-tag (11 amino acid residues), the green fluorescent protein (GFP) sequence (26 kDa), or an unrelated peptide (3 amino acid residues) to the N-terminus of the head domain does not affect lamin assembly in vitro or in vivo,12,20 the purified His-tagged mutants were applied to the subsequent in vitro assembly reactions without removal of the His-tag sequence.

**Dimer formation of full-length mouse lamin A and deletion mutants**

After dialysis against dimerization buffer (see "Materials and Methods"), full-length mouse lamin A revealed an approximately 50-nm long rod with two globules at one end (Fig. 2a), evidently representing an α-helical coiled-coil dimer made from two parallel and unstacked lamin molecules.1,9–14,35 The two globules often coalesced into one. Similarly to full-length lamin A, all deletion mutants, LamA(ΔN10), LamA(ΔN20), and LamA(ΔHD), formed an approximately 50-nm long rod with two globules at one end (Fig. 2b to d). The size and shape of these dimers were indistinguishable from that of dimers formed by full-length lamin A. These results confirm the idea that the head domain is dispensable to the formation of lamin dimer.11,12,14

**Effects of deletions on head-to-tail polymerization**

As shown in Fig. 3a, one-step dialysis of urea-solubilized full-length mouse lamin A against dimer-elongation buffer (see "Materials and Methods") for 3 h yielded short head-to-tail polymers made of lamin A dimers. These polymers grew longer during dialysis for 6 h, as shown in Fig. 3b and Fig. 4a. Among the head domain-deletion mutants, LamA(ΔN10) also formed short head-to-tail polymers by one-step dialysis for 3 h, as shown in Fig. 3c, and these polymers grew longer during dialysis for 6 h as shown in Fig. 3d and Fig. 4b.
These results suggest that the N-terminal 10 residues of the head domain are dispensable to the formation of head-to-tail polymers. The presence of the His-tag sequence did not disturb the head-to-tail polymerization. In contrast to full-length lamin A and LamA(ΔN10), LamA(ΔN20) and LamA(ΔHD) exhibited predominantly 50-nm long rods (Fig. 3e and g) indistinguishable from the products observed after dialysis against dimerization buffer (see Fig. 2c and d). These approximately 50-nm long rods did not grow longitudinally during dialysis for 6 h, as shown in Fig. 3f, h and Fig. 4c, d. Thus, LamA(ΔN20) and LamA(ΔHD) showed significantly decreased competence in assembling into head-to-tail polymers. These results suggest that the last 20 residues (from Arg-11 to Gln-30) of the head domain contain essential structural elements to the formation of head-to-tail polymers (see “Discussion”).

Effects of deletions on paracrystal formation
As shown in Fig. 5a to d, one-step dialysis of urea-solubilized full-length lamin A, LamA(ΔN10), LamA(ΔN20), and LamA(ΔHD) individually against paracrystal forming buffer (see “Materials and Methods”) yielded paracrystals. While paracrystals of full-length lamin A exhibited evenly spaced light-dark transverse bands with an axial repeat pattern of 25–26 nm (Fig. 5e), paracrystals of LamA(ΔN10), LamA(ΔN20), and LamA(ΔHD) showed light-dark transverse bands with an axial repeat pattern of 49–50 nm, in which there were

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**Fig. 4.** Length Distribution of Head-to-Tail Polymers of Full-Length Mouse Lamin A (a) and Deletion Mutants LamA(ΔN10) (b), LamA(ΔN20) (c), and LamA(ΔHD) (d).

The lengths of samples after dialysis against dimer-elongation buffer at room temperature for 3 h (white bar) and 6 h (black bar) are shown in numbers (1–5) of dimer molecules annealed longitudinally.

These results suggest that the N-terminal 10 residues of the head domain are dispensable to the formation of head-to-tail polymers. The presence of the His-tag sequence did not disturb the head-to-tail polymerization. In contrast to full-length lamin A and LamA(ΔN10), LamA(ΔN20) and the head-less mutant LamA(ΔHD) showed significantly decreased competence in assembling into head-to-tail polymers. These results suggest that the last 20 residues (from Arg-11 to Gln-30) of the head domain contain essential structural elements to the formation of head-to-tail polymers (see “Discussion”).

**Fig. 5.** Paracrystal Formation of Full-Length Mouse Lamin A (a) and Deletion Mutants LamA(ΔN10) (b), LamA(ΔN20) (c), and LamA(ΔHD) (d).

Urea-solubilized proteins (0.25 mg/ml) were dialyzed against paracrystal forming buffer at room temperature for 2–6 h, as described in “Materials and Methods.” For electron microscopy, samples were prepared by negative staining with 2% uranyl acetate, also as described in “Materials and Methods.” Note the differences between the light-dark transverse bands of full-length mouse lamin A (e) and the bands of deletion mutants LamA(ΔN10) (f), LamA(ΔN20) (g), and LamA(ΔHD) (h). Arrow shows either an axial repeat pattern of 25–26 nm containing evenly spaced light-dark transverse bands, in (e), or a repeat pattern of 49–50 nm containing two approximately 15-nm-wide light bands separated by dark bands of approximately 6 nm and 14 nm in width alternately, in (f) to (h).
two 15 nm-wide light bands separated by dark bands of 6 nm and 14 nm in width alternately (Fig. 5f to h). Similar patterns with alternating narrower and wider dark bands have been described for paracrystals of headless chicken lamin B2 mutants,11) but not for the headless human lamin C mutant 12) or the headless Drosophila lamin Dm0 mutant.14)

**Discussion**

Longitudinal head-to-tail association of lamin dimers is a critical first step in lamin IF assembly.2,7,8) Although it has been reported that the N-terminal head domain of lamins is critically involved in head-to-tail association,11,14) essential structural elements of the head domain have remained unsolved. In this study, we engineered stepwise deletions of the head domain (30 amino acid residues) of mouse lamin A from its N-terminus at intervals of 10 amino acid residues, and analyzed the assembly properties in vitro of the lamin A mutants by electron microscopy, using both glycerol spraying/low-angle rotary metal shadowing and negative staining for specimen preparation. The results obtained indicate that the last 20 residues (from Arg-11 to Gln-30) of the head domain of mouse lamin A contain structures essential to the head-to-tail association of lamin A dimers.

The head domains of nuclear lamins (Fig. 6) are relatively short as compared to the head domains of cytoplasmic IF proteins (typically 40–100 residues). In spite of such differences in length, the head domains of nuclear lamins and cytoplasmic IF proteins are basic due to a wealth of arginine residues. Acidic residues and lysine, although occasionally present, are rare. In the assembly of cytoplasmic IFs, some arginine residues in the head domain are critically involved. This has been shown by deletion via proteolysis25,26) and recombinant DNA technology 27,28) of the highly conserved sequence motif containing two consecutive arginines (-S-S-Y-R-R-X-F-G-G-) near the beginning of the head domain of cytoplasmic type-III and type-IV IF proteins, and by addition of an exogenous peptide representing the conserved sequence motif,29,30) conditions under which IF formation is inhibited or disturbed. The addition of free arginine to IF assembly reactions30) and enzymatic deimination of arginine residues in cytoplasmic IF proteins to citrulline31) also inhibited IF formation.

Therefore, it is conceivable that arginine residues in the head domain of nuclear lamins might also be involved in lamin polymerization. Whereas lamin A proteins commonly have two consecutive arginine residues near the beginning of the head domain (Fig. 6), our results indicate that the N-terminal 10 residues including the two arginines (Agr-7 and Arg-8) are not involved in the head-to-tail association of lamin A dimers. Among the five arginine residues in the head domain of mouse lamin A, Arg-11 is relatively conserved in both A- and B-type lamins (Fig. 6). In this study, deletion of the N-terminal 20 residues of the head domain of mouse lamin A resulted in loss of the potential to form head-to-tail polymers. This result suggests the possibility that Arg-11 is involved in head-to-tail polymer formation. The
other two arginine residues (Arg-25 and Arg-28) are included in the 12 highly conserved residues (-T/S-P-L-S-P-T-R-I/L-T/S-R-L-Q-, Fig. 6) located in the C-terminal region of the head domain. As expected, deletion of the whole head domain (the N-terminal 30 residues) of mouse lamin A prevented head-to-tail polymer formation. Thus our results indicate that the N-terminal two consecutive arginines (Arg-7 and Arg-8) are not involved in lamin A polymerization, and that the exact roles of Arg-11, Arg-25, and Arg-28 remain to be adequately characterized.

It has been reported that head-to-tail association of lamin dimers involves an overlap of the highly conserved N-terminal segment of coil 1A and the highly conserved C-terminal segment of coil 2B by 2–4 nm.2,6,13 Recently, Strelkov et al. resolved the crystal structure of human lamin A coil 2B dimer, and proposed an atomic model highlighting possible interactions responsible for the N to C overlap of lamin dimers.36 In their model, the acidic patch formed by the conserved C-terminal segment of coil 2B interacts electrostatically with certain arginine residues in the head domain. From our results, the three arginine residues (Arg-11, Arg-25, and Arg-28) in the head domain of mouse lamin A are candidates as sites for such electrostatic interaction.

Although the internal sequence from Ser-12 to Ser-18 is not conserved among lamin proteins, this region is commonly composed of small, hydrophilic residues (Fig. 6). At present we cannot exclude the possibility that this flexible region is important as a spacer between the conserved Arg-11 residue and the conserved C-terminal region (from Thr-19 to Gln-30), or that this region has a role in stabilizing a certain conformation of the conserved C-terminal region of the head domains of lamin. It should be examined in the future whether this internal region is involved in lamin A polymerization, using mutant proteins lacking only this region.

It is difficult to assume that the highly conserved C-terminal region from Thr-19 to Gln-30 is full enough to form head-to-tail polymers, or that, in LamA(ΔN20), the reduction of the competence to polymerize was caused principally by deletion of Thr-19 and Pro-20, since chicken lamin B1 inherently lacks these two residues (Fig. 6). Thus the highly conserved C-terminal region might play an important role in lamin polymerization, in cooperation with other residues, such as Arg-11, located at the N-terminal side. In addition, the highly conserved C-terminal region contains the phosphorylation site, Ser-22, for cdc2 kinase, which affects lamin IF organization in lamina during cell division21–23 and induces disassembly in vitro of the head-to-tail polymers.24 Cdc2 kinase is a proline-directed protein kinase that prefers phosphorylation site sequences in the following order: P-X-S(PO4)2-P-X-R > S(PO4)2-P-X-R > S(PO4)-P.37,38 Thus the sequence from Pro-20 to Arg-25 in mouse lamin A is sufficient at least in that it provides the optimal substrate recognition sequence for cdc2 kinase. Further work is required to elucidate the way the lamin A head domain sequence from Arg-11 to Gln-30 is involved in head-to-tail association. Such studies would be helpful to understand the molecular mechanism of phosphorylation-dependent disassembly of head-to-tail polymers and lamin IFs.

The lamin A mutants in this study readily formed paracrystals with light-dark transverse bands slightly different from the bands observed for full-length lamin A paracrystals. The reason for the changes in the banding patterns is currently unknown. We could not examine the effects of head domain deletions on lamin A IF formation. Establishment of a suitable condition that enables stable lamin A IF formation in vitro is required in order to determine the molecular mechanism of lamin IF assembly and disassembly.

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