Crystal structures of the coil 2B fragment and the globular tail domain of human lamin B1
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ABSTRACT
We present here the crystal structures of human lamin B1 globular tail domain and coiled 2B domain, which adopt similar folds to Ig-like domain and coiled-coil domain of lamin A, respectively. Despite the overall similarity, we found an extra intermolecular disulfide bond in the lamin B1 coil 2B domain, which does not exist in lamin A/C. In addition, the structural analysis indicates that interactions at the lamin B1 homodimer interface are quite different from those of lamin A/C. Thus our research not only reveals the diversely formed homodimers among lamin family members, but also sheds light on understanding the important roles of lamin B1 in forming the nuclear lamina matrix.

Structured summary of protein interactions:
Lamin-B and Lamin-B bind by x-ray crystallography (View interaction)

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1. Introduction
Nuclear lamins are fibrous proteins that interact with many transmembrane proteins of the inner nuclear membrane and several chromatin proteins to form the nuclear lamina on the interior of the nuclear envelope [1]. Studies, both from normal cells and cells from the multitude of laminopathy patients, show that nuclear lamins play important roles in structural function and transcriptional regulation in the cell nucleus [2]. Lamin genes are found in all metazoan cells, including mammals. Lamins are divided into A and B types based on sequence homology [3]. Two major A-type lamins (lamin A and C) are splice variants derived from the LMNA gene found at 1q21, while B-type lamins, B1 and B2, are expressed from different genes, LMNB1 and LMNB2, respectively [4–6].

Like most intermediate filament (IF) proteins, the lamins are composed of a long central α-helical rod domain, flanked by a non-α-helical N-terminal domain (head) and a globular C-terminal domain (tail) [Fig. 1A] [7]. In lamins, the rod domain is further distinguished by the long coil 1 (188 amino acids) and short coil 2 (142 amino acids) segments which are joined by a linker, L12. And coil 1 segment comprises two coiled-coil segments named 1A and 1B which are further interconnected by linker L1, while in coil 2, linker L2 joins the two coiled-coil segments 2A and 2B. (Fig. 1A) [7]. Furthermore, the C-terminal domain of all lamins contains a conserved segment of 108 amino acids that forms a globular domain adopting an immunoglobulin-like fold (Ig-fold) [8]. It has been shown that both the highly conserved N- and C-terminal segments of the rod domain are responsible for the assembly of lamins [9,10], and the globular tail domain possesses the properties typical for versatile interactions [8,11].

Recently, nuclear lamins have received a great deal of interest because mutations in lamins lead to a large number of severe human diseases and aging. Mutations in LMNA cause many diseases, including muscular dystrophies and premature aging or progeroid syndromes [12]. A few diseases associated with mutations in the LMNB1/B2 genes have also been discovered, which include autosomal-dominant leukodystrophy caused by a duplication of LMNB1, and acquired partial lipodystrophy caused by LMNB2 mutations [2]. Although it is clear that chromatin organization and histone methylation are altered in diseases caused by mutation of lamins,
Fig. 1. Crystal structure of the coil 2B segment of human lamin B1. (A) Schematic diagram of lamin B1. Rectangles denote the long central $\alpha$-helical rod domain including the coil 1 (segments 1A and 1B), and coil 2 (segments 2A and 2B). Linkers L1, L2 and L12 are shown as thin lines. The globular tail domain is shown as an oval. (B) Superposition of the coil 2B of lamin B1 (green) and lamin A (magenta). (C) Details of the coiled-coil interfaces in lamin B1 coil 2B segment. The full-atom representation and the plots of BSA/residue are shown for each interface. Residues forming the hydrophobic core of the coiled-coils are colored red within the full-atom model. (D) Cartoon representation of the crystal structure of lamin B1 coil 2B segment. The two subunits of the homodimer are colored in cyan and yellow, respectively. Hydrogen bonds are shown by black dashes, and the disulfide bond is shown by a yellow stick. (E) Sequence alignment of the coil 2B segment of human lamin B1 and lamin A. Sequences showed in different color indicate different chains in coiled-coil homodimer. Residues involved in the formation of interhelical salt bridges in lamin B1 and lamin A are marked by stars and dots, respectively, and salt bridges are shown by dashes. The cystine residue and its disulfide bond in lamin B1 are marked with a triangle and a line, respectively. The alignment was created with Espript (http://espript.ibcp.fr/Espript/Espript).
it is still unclear how lamins act in the wide range of nuclear functions [2].

Lamin A and lamin B1 exhibit high sequence identity, especially in short coil 2 and globular tail domain, which share 62% and 51% identities, respectively (Fig. 1E and Fig. 2A) [13]. In addition, they also exhibit similar structure features including coiled-coil assembly and Ig-like fold, which have been mentioned above. Thus, it is interesting that how lamins can differentiate from each other both in lamin filaments organization and substrate recognition.

Here we report two crystal structures of the coiled-coil dimer from the second half of the coil 2 segment (coil 2B: residues 311–388) and the globular tail domain (residues 428–550) of human lamin B1 at 2.4 and 2.0 Å, respectively. As expected, coil 2B segments of human lamin B1 form left-handed parallel coiled-coil homodimers. However, in contrast to the conserved conformation of the homologous segment of human lamin A, the patterns of hydrogen bonds and salt-bridge interactions in the lamin B1 coil 2B segment are not conserved in lamin A and an extra disulfide bond is formed between the N-terminus of two lamin B1 coiled-coil subunits. We also demonstrate that the crystal structure of the lamin B1 globular tail domain adopts a conserved, β-Ig-like fold with a little conformation changes at the potential substrate recognition site compared to lamin A. In summary, the structure features of both the coiled-coil assembly of coil 2B segment and the different conformation of the globular tail domain differentiate lamin B1 from other lamins, and our results provide additional insight into the biological functions of nuclear protein components.

2. Materials and methods

2.1. Protein expression and purification

The human lamin B1 globular tail domain (residues 428–550) and coil 2B segment (residues 311–388) were subcloned into the pET28a-MHL vector and the recombinant proteins were expressed and purified as described before [14]. After purification using a HiTrap Nickel column and a Superdex 75 gel-filtration column, the globular tail domain and coil 2B segment were concentrated to 15 and 20 mg/mL, respectively, in a buffer containing 20 mM Tris–HCl, pH 7.5, 0.15 M NaCl and 1 mM DTT.

2.2. Crystallization

The purified globular tail domain and coil 2B segment of lamin B1 were crystallized using the sitting drop vapor diffusion method at 18 °C. Diffraction-quality crystals of the globular tail domain of lamin B1 were grown in a buffer containing 0.1 M Tris–HCl, pH 8.5, 0.2 M MgCl2, and 30% PEG4000. The crystals were cryoprotected using a mixture consisting of 85% reservoir solution and 15% glycerol (v/v). Crystals of the human lamin B1 coil 2B segment were grown at 18 °C using the hanging drop method by mixing equal volumes of protein and the reservoir solution containing 0.1 M bis–Tris–HCl, pH 6.5, 0.1 M CrCl3, 0.2 M LiSO4, and 25% PEG3350. The crystals were cryoprotected by a solution consisting of reservoir solution with 20% glycerol (v/v).

2.3. Data collection, structure determination and refinement

Diffraction data were collected at 100 K on beamline 23-ID-B (GM/CA-CAT, Advanced Photon Source, Argonne National Laboratory) and data were integrated and scaled using the HKL2000 software package [15]. The structures of coil 2B segment and the globular tail domain of lamin B1 were solved using automated molecular replacement algorithms as implemented by MrBUMP and using PHASER in the CCP4 program suite [16]. The structures of human lamin A coil 2B segment (PDB 1X8Y) and lamin A/C globular domain (PDB 1IFR) were used as search models, respectively. Following several alternate cycles of manual rebuilding using

![Fig. 2. Crystal structure of the globular tail domain of lamin B1. (A) Sequence alignment of the globular tail domain of human lamin B1 and lamin A. Aromatic residues that lie in the groove between the β-sheets are marked by dots. The alignment was created with Esprit (http://esprit.ibcp.fr/Esprit/Esprit). (B) Superposition of the globular tail domain of lamin B1 (green) and lamin A (magenta). (C) Structure of the lamin B1 globular tail domain. (D) Superposition of the aromatic residues that lie in the groove between the β-sheets. Residues are shown in stick mode. Lamin B1 and lamin A are colored green and magenta, respectively. (E) Superposition of lamin B1 (green) and lamin A (magenta) at the groove between the two β-sheets and the β7β8 loop.](http://example.com/fig2)
The quality of the X-ray diffraction data and the structure refinement parameters are shown in Table 1. The structures were solved using molecular replacement. The final cycles of model building, TLS parameterization was performed using REFMAC [18] in the CCP4 program suite [16]. During the final cycles of model building, TLS parameterization was included in the refinement of the final model, which comprised protein and solvent molecules. Data collection and refinement statistics are summarized in Table 1.

### 3. Results and discussion

The crystals of the coil 2B segment and the globular tail domain of human lamin B1 diffracted to 2.4 and 2.0 Å resolution, respectively. The structures were solved using molecular replacement. The quality of the X-ray diffraction data and the structure refinement parameters are shown in Table 1.

Our crystal structure indicates that coil 2B segments of human lamin B1 form a left-handed parallel coiled-coil homodimer (Fig. 1B and C). Consistent with the high sequence similarity, the overall conformation of the lamin B1 coil 2B dimer is similar to that of human lamin A coil 2B [19]. Superposition of the lamin B1 coiled-coil dimer (residues 313–382) onto the corresponding residues of lamin A coiled-coil dimer (PDB:1X8Y, residues 313–386) yields a root-mean-square deviation of 1.69 Å for the Cα atoms (Fig. 1B). In addition, the total buried surface area (BSA) of the coiled-coil interface formed by lamin B1 coil 2B segments was calculated to be 3765 Å², also comparable to that of the lamin A coil 2B dimer (3562 Å²).

We then analyzed in detail the interactions contributing to the formation of the coiled-coil homodimer. As expected, the patterns of the residues contributing to the hydrophobic core of the interface of the coiled-coil homodimer are similar to that of the left-handed parallel interface in lamin A [305–387] segment [13]. The hydrophobic core residues follow the same heptad pattern with the a and d positions of the heptad forming the core, which have been described [13,19] (Fig. 1C). Besides the numerous of hydrophobic interactions, there is also an extensive network of hydrogen bonds and salt bridges at the homodimer interface. Different from lamin A, more interhelical hydrogen bonds are formed at the coiled-coil homodimer interface of lamin B1-10 in lamin B1 compared to 4 in lamin A (Fig. 1D). In addition, intermolecular salt bridges also play an important role in the formation of the homodimer. Some of them are conserved in the lamin A coil 2B coiled-coil dimer, such as the one between Glu343 and Lys342, whereas others only exist in the lamin B1 coil 2B coiled-coil dimer. As expected, the patterns of the residues contributing to the hydrophobic core of the homodimer (Fig. 1D and E), which is not present in either vimentin (PDB:1GK4) or lamin A/C coiled-coils (PDB: 1X8Y and 2XV5). Accordingly, we believe that a number of hydrogen bonds and salt bridges, as well as the unexpected disulfide bond, in the lamin B1 coil 2B coiled-coil specify the coiled-coil architecture of lamin B1 coil 2B segment.

The crystal structure of the lamin B1 globular tail domain (residues 428–550) reveals that this C-terminal domain adopts an Ig-like fold, which consists of a 2-layered sandwich of 9 anti-parallel β-strands arranged in two β-sheets with a Greek key topology. One of the sheets has five β-strands while the other has four. Two short bridge-like strands (residues 464–465 and 489–490) are present and link the two β-sheets at the top. Short loops connect most of the β-strands, leading to the compact appearance of the domain (Fig. 2A–C).

The two sheets of the β sandwich in the lamin B1 globular tail domain are associated closely. The densely packed core is formed by residues from β-strands β2 to β9, bridge-like strands β3' and β5', and loops between β3/4, β5/6 and β7/8 (Fig. 2C). The longest loop β7/8 wraps around the domain and fills the cleft where the β-sheets meet (Fig. 2C). Two grooves, which have been considered as a potential site for intermolecular interactions [8], are formed on the domain surface, the larger one between β-strands β7 and β5 with aromatic residues Tyr482, Tyr484, Tyr488 and Trp515 at its base and the smaller one between loop β7/8 and β sheet β3-5 (Fig. 2C and D). The crystal packing between two Ig-like domains of lamin B1 is not representative of the actual interaction between lamin B1 Ig-like domains, which behave as monomers in solution.

Based on the high sequence homology of lamin B1 with lamin A (Fig. 2A), the overall structure of the lamin B1 globular tail domain is similar to the structure of lamin A (PDB: 1IFR), as expected [8]. Comparison of the structures of the globular tail domains of lamin B1 (residues 431–548) and the corresponding segment of lamin A (residues 432–544) reveals a root-mean-square deviation of 0.68 Å superposed over all of their overlapping Cα atoms (Fig. 2B). In spite of the overall conformational similarity, there are variations in the attachment around the β-sandwich fold. Though the aromatic residues Tyr482, Tyr484 and Tyr488 lying in the larger groove formed between β strands β7 and β5 superpose very well with the homologous residues in the structure of the lamin A tail domain, the two other aromatic residues Trp515 and Trp521 belonging to the β7/8 loop do not coincide very well with the homologous ones of lamin A.

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a The values in parentheses refer to statistics in the highest shell.

b Rmerge = 1/ | | / | |, where | | is the intensity of the ith measurement, and  is the mean intensity for that reflection.

c Rmerge = Σi(|Fi(h)−|Fobs(h))/Σi|Fi(h)|, where F0 and Fi are the observed and calculated structure factor amplitudes, respectively.

d Rwork was calculated with 10% of the reflections in the test set.

e Categories were defined by Procheck.
A (Fig. 2D). Because of this, the β7β8 loop deviates from its counterpart in lamin A by 3.8–5.1 Å (Cα of Gly522, Thr523 and Gly524) (Fig. 2E). The migration of the β7β8 loop and the two aromatic residues accordingly affect the size and the conformation of the two grooves. These grooves probably act as a recognition site, and therefore determine the substrate specificity of lamin B1 and distinguish it from other proteins that contain Ig-like domains.

4. Conclusion

We propose that the architectural features of the coiled-coil, especially the intermolecular disulfide bond found only in the lamin B1 coil 2B segment and not in lamin A or vimentin, along with the pattern of the β7β8 loop which inserts into the clefts where the two β-sheets meet, differentiate lamin B1 from other nuclear lamins.

5. PDB accession numbers

Atomic coordinate and structure factors of human lamin B1 coil 2B fragment and the globular tail domain were deposited in the Protein Data Bank under accession numbers 3TYY and 3UMN, respectively.

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