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Lateral A₁₁ type tetramerization in lamins

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Abstract

The assembly of intermediate filaments (IFs) including nuclear lamins is driven by specific interactions of the elementary coiled-coil dimers in both lateral and longitudinal direction. The assembly mode A₁₁ is dependent on lateral tetramerization of the second coiled-coil segment (coil1b) in antiparallel fashion. Recent cryo-electron microscopy studies pointed to 3.5 nm lamin filaments built from two antiparallel threads of longitudinally associated dimers but little molecular detail is available to date. Here we present the 2.6 Å resolution X-ray structure of a lamin A fragment including residues 65 to 222 which reveals the molecular basis of the A₁₁ interaction. The crystal structure also indicates a continuous α -helical structure for the preceding linker L1 region. The middle part of the antiparallel tetramer reveals unique interactions due to the lamin-specific 42-residue insert in coil1b. At the same time, distinct characteristics of this insert provide for the preservation of common structural principles shared with lateral coil1b tetramers of vimentin and keratin K1/K10. In addition, structural analysis suggests that the A₁₁ interaction in lamins is somewhat weaker than in cytoplasmic IFs, despite a 30% longer overlap. Establishing the structural detail of the A₁₁ interaction across IF types is the first step towards a rational understanding of the IF assembly process which is indispensable for establishing the mechanism of disease-related mutations.

Keywords: intermediate filaments, nuclear lamins, coiled coil, X-ray crystallography

Abbreviations: IF, intermediate filament; ATP, adenosine triphosphate; PEG, polyethylene glycol; r.m.s.d., root mean-square deviation

Introduction

Intermediate filament (IF) protein family includes about 70 members in humans (Herrmann et al., 2002). These filaments have distinct tissue localizations and varied specific functions centred around their paradigmatic structural and mechanical capacity (Herrmann et al., 2007). While most IF proteins are found in the cytoplasm, lamins represent a special IF class forming the nuclear lamina, a network-like structure attached on the inside of the nuclear membrane (Aebi et al., 1986; Dwyer and Gunter, 1976; Gerace et al., 1978). Lamins are of particular interest due to their association with a large number of inherited mutations that cause neurological and muscular disease as well as premature ageing (Butin-Israeli et al., 2012; Dechat et al., 2008; Goldman et al., 2004; Östlund et al., 2001).

The elementary building block of all IFs is an elongated dimer based on a coiled-coil structure (Lupas, 1996; Testa et al., 2009). The latter is produced by the central α -helical domain which is conserved across all IF proteins and serves as a signature of this vast protein family. Specifically, primary structure alignment of this central 'rod' domain across all IF proteins reveals three segments predicted to be continuously α -helical, termed coil1a, coil1b and coil2 (Chernyatina et al., 2015). Each of these regions reveals a hydrophobic pattern containing mostly 7-residue (heptad) repeats as well as several 11-residue (hendecad) repeats. These repeat types induce either a left-handed or a parallel (untwisted) coiled-coil structure respectively (Gruber and Lupas, 2003; Parry, 2006). In particular, the N-terminal portion of the coil2 segment contains multiple hendecad repeats, while there is another isolated hendecad repeat (equivalent to a heptad followed by a four-residue 'stutter' insert) closer to the C-terminus of coil2 (Strelkov et al., 2002b). Importantly, within each of the three segments of the rod, the sequences of all IF proteins align with a perfect preservation of the hydrophobic pattern without any insertions and deletions, with one important exception. This exception is the 42-residue (*i.e.* six heptad long) insert within coil1b that is present in nuclear lamins but not found in other IFs (Fig. 1). In contrast, the linkers L1 and L12 interconnecting the coiled-coil segments as well as the N-terminal head and C-terminal tail domains are highly variable in length and composition across different IF proteins. A particular feature of lamins is that their linker L1 is in principle compatible with a continuous coiled-coil structure from coil1a into coil1b as the corresponding heptad phase shift is equivalent to one residue *i.e.* a double stutter (Chernyatina et al., 2015) (Fig. 1).

Assembly into 10-12 nm wide filaments is a characteristic feature of cytoplasmic IF proteins. The assembly process does not require any explicit energy source like ATP and does not depend on any co-factors, at least *in vitro* where it can be induced by simply increasing the ionic strength. The driving force of assembly are specific interactions of the dimers involving both the rod and the terminal domains. Chemical cross-linking studies on assembled cytoplasmic filaments of vimentin, keratins and neurofilament proteins have consistently suggested three specific modes of lateral association of dimers (Parry and Steinert, 1999; Steinert et al., 1993b, 1993a). Specifically, the coiled-coil dimers were reported to arrange antiparallel to each other with three distinct staggering possibilities, namely the A_{11} mode corresponding to a register of the coil1b segments, the A_{22} mode aligning the coil2 segments, and the A_{12} mode aligning the complete rod domains. In addition, a linear alignment of two equally oriented dimers one after the other with a short overlap corresponded to the A_{CN} mode which contributes to the filament assembly in the longitudinal direction. It was also shown that cytoplasmic IF proteins produce tetramers based on the A_{11} interaction already in solution. Further lateral assembly of tetramers yields

the so-called unit-length filaments (Herrmann et al., 1996; Mücke et al., 2004) which associate in the longitudinal direction.

Nuclear lamins exhibit a somewhat different assembly pathway. Early on, *in vitro* they were seen to yield longer thin threads of dimers all oriented in the same way, suggesting that a longitudinal association of dimers was a dominant feature (Aebi et al., 1986; Geisler et al., 1998; Heitlinger et al., 1991). Just like for other IF types, the intrinsically disordered head and tail domain play a key role in lamin assembly (Heitlinger et al., 1992). Filamentous structures composed of two antiparallel, laterally associated long dimer threads (thus counting four lamin chains per cross-section) were also present. In addition, further lateral assembly was observed, resulting in paracrystals (Ben-Harush et al., 2009; Heitlinger et al., 1991; Stuurman et al., 1998). In general, the appearance of various *in vitro* assembled structures was dependent on the conditions used such as pH and ionic strength (Foeger et al., 2006; Geisler et al., 1998). Recent studies of *ex vivo* lamina using cryo-electron tomography pointed to 3.5 nm lamin filaments which were also interpreted as being made of two antiparallel dimeric threads (Turgay et al., 2017).

Even though crystallization of full-length IF proteins is generally considered impossible, crystallographic studies of IF protein fragments have been instrumental in obtaining the molecular detail at the dimer and more recently tetramer level. For lamins, crystallographic studies have thus far been focused on the C-terminal portion of the rod domain as well as the globular part of the tail not directly involved in filament assembly (Dhe-paganon et al., 2002; Kapinos et al., 2010; Strelkov et al., 2004). Here we report the atomic structure of a lamin A fragment encompassing residues 65 to 222 and thus corresponding to the linker L1 and the coil1b domain. This fragment reveals a continuous dimeric coiled-coil structure including the linker. Most interestingly, the fragments interact laterally in a way corresponding to the A₁₁ assembly mode. Here we compare the molecular detail of this interaction to that has previously been observed for vimentin and keratin fragments. We show that the lateral antiparallel association of coil1b segments in lamins and cytoplasmic IF proteins shares common structural features, while the distinct length of this segment in lamins provides for an additional, unique interdimer contact.

Materials and methods

Cloning, purification and crystallization screening of lamin fragments were described in our previous publication (Chernyatina et al., 2016). Briefly, the fragments were overexpressed in *E. coli* BL21 (DE3) cells with an N-terminal 6xHis tag. After affinity purification, the tag was removed using TEV protease. The final purification step was performed by gel filtration chromatography. Optimized crystals of lamin A fragment 65-222 were grown using 10% PEG 8000, 0.1 M imidazole-HCl buffer (pH8.0) and 0.2 M calcium acetate as precipitant and mounted on cryo-loops using mother liquor supplemented with 25% glycerol. X-ray diffraction data were collected using beamline Proxima 1 at Synchrotron Soleil (France) equipped with a Pilatus 6M detector and an X-ray wavelength of 1.07 Å. Complete diffraction data set was obtained through standard processing in XDS (Kabsch, 2010) to 2.6 Å resolution (Table 1). Molecular replacement was carried out with coil1b dimer models obtained using the CCFold web server at <https://pharm.kuleuven.be/apps/biocryst/ccfold.php> (Guzenko and Strelkov, 2018). The initial

solution was further improved using Buccaneer (Cowtan, 2006) and Coot (Emsley et al., 2010). The same diffraction data were additionally subjected to the STARANISO algorithm (Tickle et al., 2018), resulting in a 2.4 Å dataset with partial completeness between 2.6 and 2.4 Å. These anisotropy-corrected data were used to produce improved maps towards tracing the less ordered terminal regions of the coiled-coil dimers. Initial real-space refinement of these regions was done in Coot (Emsley et al., 2010) using Ramachandran restraints corresponding to α -helical geometry. Automated refinement was carried out using Buster (Bricogne et al., 2017). The final coordinates and structure factors have been deposited to the Protein Data Bank under accession code 6SNZ. Interdimeric contacts in crystal structures have been analyzed using the program PISA (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html, Krissinel and Henrick, 2007). Coiled-coil geometry was analysed using the Twister web server at <https://pharm.kuleuven.be/apps/biocrystr/twister.php> (Strelkov et al., 2002a). Pymol (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC) was used for further structural analysis as well as for preparing figures.

Results and Discussion

Molecular structure of lamin coil1a dimer

Sequence alignment of various IF types, supplemented with α -helicity predictions and analysis of the hydrophobic repeat patterns, allows to delineate the likely borders of the consensus coil1b segment (Fig. 1A). For lamin A, this segment is predicted to start at residue 79 and end at residue 222 (144 amino acids in total). Importantly, optimal sequence alignment brings into register the distal portions of predicted coil1b segment in lamins and in cytoplasmic IF proteins, while the extra 42 residues in lamins appear as an insert in the middle of such alignment (Fig. 1A). In fact lamins are considered as the precursors of other IF types as suggested by multiple sequence alignment (Blumenberg, 1989; Guzenko et al., 2017; Peter and Stick, 2015). Moreover, lamins are even present in many protozoan organisms, revealing a remarkable sequence conservation with respect to metazoan lamins (Kollmar, 2015). Extrapolating these arguments, the shorter coil1b in cytoplasmic IF proteins should be logically considered a result of a 42-residue deletion with respect to nuclear lamins.

To approach the atomic detail of the A₁₁ assembly mode (Fig. 1B), we have created a series of recombinant lamin A constructs centred on the coil1b segment and subjected them to high-throughput crystallization screening (Chernyatina et al., 2016). The fragment including residues 65 to 222 could be used to grow crystals which yielded a complete 2.6 Å diffraction dataset. Phasing of the data using molecular replacement was initially unsuccessful, which is commonplace for coiled-coil structures (Chernyatina et al., 2016). By trying a series of coil1b segment models of various length produced by the CCFold algorithm (Guzenko and Strelkov, 2018) we could eventually obtain the right solution, which allowed further model refinement (Table 1). The crystal structure contains two coiled-coil dimers per asymmetric unit (Supplementary Fig. 1A). The AB dimer is well ordered all along its length including the predicted linker L1, while the dimer CD has some disorder near the N-terminus in both chains so that a reliable electron density only starts with the coil1b segment. Superposition of the two dimers reveals a good structural match for the predominant central part, evident from a root mean-square deviation

(r.m.s.d.) of 0.76 Å for 242 C α positions after outlier rejection as implemented in Pymol (Supplementary Fig. 1B).

As seen in Fig. 2A, the structure is completely α -helical and forms a coiled coil all along its length. Overall, the lamin coil1b segment is about 21 nm long, which is 6 nm longer than the corresponding segment in cytoplasmic IFs due to the 42-residue insert. The structure is held together by hydrophobic core residues which follow a perfect heptad periodicity starting with the N-terminus of coil1b and up to the residue 210, just as predicted previously from the amino-acid sequence (Herrmann and Aebi, 2004). In line with this, analysis with the program Twister (Strelkov et al., 2002a) (Fig. 2B) reveals a uniform left-handed coiled-coil geometry for the predominant central part of the structure (residues 100 to 200). The coiled-coil radius averages 4.8 Å within this part and the coiled-coil twist is close to -4° per residue, which corresponds to a pitch of about 145 Å. These radius and twist values are typical for heptad-based coiled-coil dimers. The region centred at residue 190 reveals an exceptionally low coiled-coil radius value of 4.3 Å (Fig. 2B). This corresponds to a particularly tight coiled-coil core packing, which appears to be linked to a cross-chain salt bridge formed between consecutive core residues Glu186 (heptad position *d*) and Arg190 (position *a*), followed by the small Ala193 residue in the next *d* position (Fig. 1A). Past residue 210, however, there is a single stutter insert which disturbs the regular heptad pattern. Accordingly, towards the C-terminus of coil1b the coiled-coil twist gradually approaches zero meaning that the two helices become nearly parallel. The latter structural feature is conserved across various IF types, as suggested by the sequence alignment (Fig. 1A), and also observed in the crystal structures obtained for the coil1b fragments of vimentin (Aziz et al., 2012; Pang et al., 2018) and keratin K1/K10 (Eldirany et al., 2019).

Lamin linker L1 preserves a continuous α -helical structure running from coil1a through coil1b

The N-terminal part (residues 67 through 78) of the resolved lamin fragment corresponds to the predicted linker L1. Importantly, also this part is completely α -helical (Fig. 2C). This part reveals an increased coiled-coil radius of between 5 and 6 Å as well as an essentially untwisted structure *i.e.* two parallel α -helices (Fig. 2B). Moreover, the residues Val70 and Val74 follow a regular hydrophobic core spacing. Except for a larger separation of α -helices, this geometry resembles the parallel α -helical bundle at the C-terminus of coil1b.

It should be noted that the sequence of L1 in lamins is indeed compatible with a smooth α -helix as it does not contain any Pro residues. In contrast, the L1 sequences of many cytoplasmic IFs such as both type I and II keratins in particular contain prolines and also vary considerably in length and amino-acid composition (Smith et al., 2002). Moreover, in lamin the phase difference between the (regular) heptad periodicity within coil1a and the (regular) coiled-coil periodicity starting with coil1b is +1 residue, which corresponds to two stutter inserts. Such inserts can be tolerated in continuous coiled coils but require a local untwisting (Chernyatina et al., 2012) just like seen near the C-terminus of coil1a in all IF proteins.

These considerations together with the observed crystal structure lead to a conclusion that within a full-length lamin dimer there would be a continuous α -helix running from coil1a into coil1b. Of note, both the L1 and coil1b parts of the crystal structure reveal an excellent match to the *in silico* model of complete lamin dimer produced with the CCFold algorithm (Guzenko and Strelkov, 2018). At the same

time, recent crosslinking experiments with full-length lamin A suggested that the structure could be more dynamic (Makarov et al., 2019). This study indicated that multiple parts of the rod domain including L1 are capable of ‘compacting’ in a zigzag fashion, ultimately enabling a spring-like action of the rod domain which may be physiologically relevant.

Lamin coil1b forms a lateral antiparallel tetramer

The two lamin fragment dimers in the crystallographic asymmetric unit run parallel to each other, with a lateral stagger of about 4 nm (Supplementary Fig. 1A). However, these two dimers only weakly interact with each other. Their interface measures 757 Å² and the solvation-associated ΔG of this interface as estimated by program PISA (Krissinel and Henrick, 2007) is positive (Table 2, interface AB/CD). In the crystals each dimer also interacts with a symmetry-related copy of another dimer which runs in the opposite direction. While such an antiparallel arrangement would in principle be consistent with the alignment of coil1b segments expected in the native 3.5 nm filaments (Turgay et al., 2017), this interface does not appear to be stable by PISA analysis (Table 2, interface AB/C'D'; the prime indicates a symmetry-related chain). At the same time, the application of crystallographic symmetry results in alternating continuous sheets formed by each of the dimers AB and CD individually (Supplementary Fig. 1C). This is due to a crystallographic two-fold screw axis that locates in the plane of the sheet and perpendicular to the dimer axis. Within each sheet, the adjacent dimers are again antiparallel to each other. Importantly, we found that the interfaces AB/A'B' and CD/C'D' within the respective sheets represent the same alignment of coil1b segments. The two tetramers can be superimposed with an r.m.s.d. of 1.78 Å for 552 C α positions after outlier rejection, with the largest deviations seen in the distal parts (Supplementary Fig. 1D). Importantly, the interfaces AB/A'B' and CD/C'D' have much larger interface areas than any other interdimer contacts in the lattice, and moreover only these two interfaces are predicted to have negative solvation-associated ΔG values, just like the yet stronger intradimer interfaces corresponding to the hydrophobic seam of the coiled coil (Table 2).

These features suggest that the observed in-register, antiparallel alignment of lamin coil1b dimers corresponds to a specific and biologically relevant interface. Crucially, as will be discussed below, such association bears distinct similarities with the coil1b tetramers of vimentin (Aziz et al., 2012; Chernyatina et al., 2012; Pang et al., 2018) and also keratin K1/K10 (Eldirany et al., 2019) that have been previously characterized using crystallography. For a vimentin coil1b fragment (PDB code 3UF1, resolution 2.8 Å), a single such tetramer was found in the asymmetric unit and no continuous sheets were present. However, crystals of the corresponding keratin K1/K10 fragment (PDB code 6ECO, resolution 3.0 Å) contained only one dimer per asymmetric unit and the tetramers were part of a continuous sheet, which is principally equivalent to the one we observed for the lamin fragment.

Common architecture of the antiparallel coil1b tetramers and lamin-specific features

The observed tetramer has two lamin A coil1b dimers nearly aligned in an antiparallel fashion with a small stagger of 1.6 nm. Interestingly, the beginning of coil1b in one dimer is aligned with the end of the regular structure with heptad repeats (residue 210) in another dimer, while the overhang corresponds to the parallel α -helical bundle part of the dimer (Fig. 3A). We have also compared our

structure with the available coil1b tetramers of vimentin and keratin K1/K10, which are quite superimposable with each other (Fig. 3A). Despite the coil1b segment in these proteins being ~30% shorter than in lamin, also here the overhanging parts correspond to the C-terminal parallel bundle.

Notably, a closer look at the nuclear and cytoplasmic coil1b tetramers reveals parallel structural features when it comes to the homologous parts in their sequence alignment (Fig. 1A). In particular, the first half of the regular heptad-based part of coil1b in keratin and vimentin (6.5 heptads) corresponds to the first 6.5 heptads in the lamin structure, while the second half of the former corresponds to the last 6.5 heptads in lamin. In all tetrameric structures, the N-terminal 6.5-heptad long regions of the dimer are nearly perfectly aligned with the C-terminal ones, which run in the opposite direction (Fig. 3A). Moreover, a structural superposition of these aligned parts in the three tetramers reveals a well conserved architecture of four α -helices (Fig. 3B). We also note that in the primary structure alignment used (Fig. 1A) the lamin-specific insert in coil1b (6 heptads) comes right in the middle of the regular heptad-based part in cytoplasmic IF proteins. Structurally this insert corresponds to ~1/2 of a superhelix turn (Fig. 3A). It is this feature that provides for the abovementioned conserved relative position of the distal parts of the coiled coils in all tetramers.

Next, detailed analysis of the interdimer interactions within the vimentin tetramer (Fig. 4A) reveals a direct contact between the first five and the last five heptads of the regular part. This interaction interface is conserved in lamin. In the keratin heterotetramer, the interaction is essentially all along the length of the dimer. Interestingly, both the left and the right half of the symmetric tetramer feature a contact between the N-terminal part of a K1 chain and the C-terminal part of a K10 chain (but not *vice versa*, see Fig. 4A). In addition, there is a hydrophobic 'knob' near the very N-terminus of one keratin K1 chain which inserts into a 'hole' formed at the C-terminus of the keratin K1 chain in another heterodimer (Eldirany et al., 2019) (Fig. 4A). The same interaction is seen in vimentin and also implied in type IV IF proteins by homology (Eldirany et al., 2019). Our structure reveals the conservation of both knob and hole elements in lamins, in line with the sequence alignment (Fig. 1A). However, due to some differences in the arrangement of the four helices near the ends of the tetramer compared to vimentin and keratin (Fig. 3B), no tight knob/hole interaction is observed in lamins (Fig. 4A).

A special feature observed in the lamin tetramer is the additional contact between the middle parts of the dimers resulting from the sequence insert. Interestingly, as indicated in Fig. 4B the two interacting, antiparallel α -helices come as much as 1.2 Å closer to each other than the parallel α -helices within the coiled coils. Such an intimate contact is possible due to two small alanine residues in positions 139 and 146. Together with residues Asn142 and Thr150 forming hydrogen bonds, these alanines enable a zipper-like arrangement (Fig. 4B).

Overall, the interdimer interface in lamin tetramer was found to be slightly smaller in area (1574 Å²) compared to both vimentin and keratin tetramers (Table 3). This corresponds to a somewhat more loose association of the dimers, given the extra length of coil1b and the additional contact provided by the central insert (~20% of the total area). In addition, structural analysis using the program PISA provides an insight into the relative strength of tetramerization. The estimated solvation energy gain upon the interface formation (ΔG) is comparable for lamin and vimentin but three times higher for keratin. The keratin interface is considerably more hydrophobic which is evident from a lower P-value (Table 3 and Supplementary Fig. 2). In addition, the lamin tetramer is stabilized by 10 H-bonds and 10 salt bridges linking the two dimers. This is comparable to keratin tetramer (12 H-bonds / 4 salt bridges)

but substantially fewer than in vimentin (20/20, Table 3). Based on these observations, we conclude that the lateral coil1b interaction is somewhat weaker in lamins compared to both keratins and vimentin.

Conclusions

For cytoplasmic IF proteins, the alignment of coil1b dimers in antiparallel fashion is known to drive the A_{11} type association of the elementary dimers, which also results in stable tetramers in low ionic strength solutions (Herrmann et al., 1996). Such alignment was well studied in the past using chemical crosslinking for both vimentin and keratins (Parry and Steinert, 1999; Steinert et al., 1993b, 1993a), as well as confirmed using spin labelling and electron paramagnetic resonance (Hess et al., 2004). The crystal structures of isolated coil1b tetramers of vimentin and keratin K1/K10 provided molecular detail of this interaction. For nuclear lamins, the A_{11} interdimer contact, alongside with the A_{22} contact aligning the antiparallel coil2 segment and the A_{CN} contact driving the longitudinal assembly, have been implied in the recent models of the 3.5nm filament (Ahn et al., 2019; Makarov et al., 2019; Turgay et al., 2017). However, a recent crosslinking study of *ex vivo* lamin filaments (Makarov et al., 2019) did not yield the exact register of two aligned coil1b segments.

Here we have presented the molecular detail of the coil1b tetramerization that is the basis of the A_{11} assembly in lamins. We show that there is a remarkable commonality in the overall architecture of the antiparallel tetramer between the cytoplasmic and nuclear IF proteins. Notably, the lamin-specific sequence insert in coil1b reveals multiple features that make this possible. First, the insert preserves the heptad periodicity, which provides for a smooth coiled-coil dimer formation. Second, the insert length (6 heptads) corresponds to one-half of a characteristic superhelical turn. In a homodimer context, this provides for an equivalent alignment of the distal regions of two antiparallel coil1b segments as in cytoplasmic proteins. Finally, a unique sequence pattern near the middle of the insert provides for an additional interdimer contact. At the same time, structural analysis indicates that despite a 30% longer coil1a segment, the lateral A_{11} type association appears less tight in lamins compared to cytoplasmic IF proteins. Ultimately this may explain the formation of thinner lamin filaments compared to the typical 10-12nm cytoplasmic filaments.

Our understanding of the molecular basis of the A_{11} interaction mode thus becomes rather detailed across various IF types. However, the two other principal modes A_{22} and A_{CN} are still awaiting more research, as no crystal structures of these assemblies for any IF protein are available to date. Such atomic level information is indispensable towards a rational understanding of disease-related mutations in various IF proteins which largely remains beyond reach until now despite their importance (de Leeuw et al., 2018; Eriksson et al., 2009; Herrmann et al., 2007; Omary, 2009; Schietke et al., 2006).

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References

- Aebi, U., Julie, C., Buble, L., Gerace, L., 1986. The nuclear lamina is a meshwork of intermediate-type filaments. *Nature* 324, 698–699. <https://doi.org/10.1038/320129a0>
- Ahn, J., Jo, I., Kang, S., Hong, S., Kim, S., Jeong, S., Kim, Y.-H., Park, B.-J., Ha, N.-C., 2019. Structural basis for lamin assembly at the molecular level. *Nat. Commun.* 10, 3757. <https://doi.org/10.1038/s41467-019-11684-x>
- Aziz, A., Hess, J.F., Budamagunta, M.S., Voss, J.C., Kuzin, A.P., Huang, Y.J., Xiao, R., Montelione, G.T., Fitzgerald, P.G., Hunt, J.F., 2012. The Structure of Vimentin Linker 1 and Rod 1B Domains Characterized by Site-directed Spin-labeling Electron Paramagnetic Resonance (SDSL-EPR) and X-ray Crystallography. *J. Biol. Chem.* 287, 28349–28361. <https://doi.org/10.1074/jbc.M111.334011>
- Ben-Harush, K., Wiesel, N., Frenkiel-Krispin, D., Moeller, D., Soreq, E., Aebi, U., Herrmann, H., Gruenbaum, Y., Medalia, O., 2009. The Supramolecular Organization of the *C. elegans* Nuclear Lamin Filament. *J. Mol. Biol.* 386, 1392–1402. <https://doi.org/10.1016/j.jmb.2008.12.024>
- Blumenberg, M., 1989. Evolution of homologous domains of cytoplasmic intermediate filament proteins and lamins. *Mol. Biol. Evol.* 6, 53–65. <https://doi.org/10.1093/oxfordjournals.molbev.a040533>
- Bricogne, G., Blanc, E., Brand, M., Flensburg, C., Keller, P., Paciorek, W., Roversi, P., Sharff, A., Smart, O.S., Vonrhein, C., Womack, T.O., 2017. BUSTER. Cambridge, United Kingdom Glob. Phasing Ltd.
- Butin-Israeli, V., Adam, S.A., Goldman, A.E., Goldman, R.D., 2012. Nuclear Lamin Functions and Disease. *Trends Genet.* 28, 464–471. <https://doi.org/10.1016/j.tig.2012.06.001>
- Chernyatina, A.A., Guzenko, D., Strelkov, S. V., 2015. Intermediate filament structure: The bottom-up approach. *Curr. Opin. Cell Biol.* 32, 65–72. <https://doi.org/10.1016/j.ceb.2014.12.007>
- Chernyatina, A.A., Hess, J.F., Guzenko, D., Voss, J.C., Strelkov, S. V., 2016. How to Study Intermediate Filaments in Atomic Detail, 1st ed, *Methods in Enzymology*. Elsevier Inc. <https://doi.org/10.1016/bs.mie.2015.09.024>
- Chernyatina, A.A., Nicolet, S., Aebi, U., Herrmann, H., Strelkov, S. V., 2012. Atomic structure of the vimentin central α -helical domain and its implications for intermediate filament assembly. *Proc. Natl. Acad. Sci. U. S. A.* 109, 13620–13625. <https://doi.org/10.1073/pnas.1206836109>
- Cowtan, K., 2006. The Buccaneer software for automated model building. 1. Tracing protein chains. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 62, 1002–1011. <https://doi.org/10.1107/S0907444906022116>
- de Leeuw, R., Gruenbaum, Y., Medalia, O., 2018. Nuclear Lamins: Thin Filaments with Major Functions. *Trends Cell Biol.* 28, 34–45. <https://doi.org/10.1016/j.tcb.2017.08.004>
- Dechat, T., Pflughaar, K., Sengupta, K., Shimi, T., Shumaker, D.K., Solimando, L., Goldman, R.D., 2008. Nuclear lamins: Major factors in the structural organization and function of the nucleus and chromatin. *Genes Dev.* 22, 832–853. <https://doi.org/10.1101/gad.1652708>
- Dhe-paganon, S., Werner, E.D., Chi, Y.I., Shoelson, S.E., 2002. Structure of the globular tail of nuclear lamin. *J. Biol. Chem.* 277, 17381–17384. <https://doi.org/10.1074/jbc.C200038200>
- Dwyer, N., Gunter, B., 1976. A modified procedure for the isolation of a pore complex-lamina fraction from rat liver nuclei. *J. Cell Biol.* 70, 581–591. <https://doi.org/10.1083/jcb.70.3.581>
- Eldirany, S.A., Ho, M., Hinbest, A.J., Lomakin, I.B., Bunick, C.G., 2019. Human keratin 1/10-1B tetramer structures reveal a knob-pocket mechanism in intermediate filament assembly. *EMBO J.* 1–19.

- <https://doi.org/10.15252/emj.2018100741>
- Emsley, P., Lohkamp, B., Scott, W.G., Cowtan, K., 2010. Features and development of Coot. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 66, 486–501. <https://doi.org/10.1107/S0907444910007493>
- Eriksson, J.E., Dechat, T., Grin, B., Helfand, B., Mendez, M., Pallari, H.-M., Goldman, R.D., 2009. Introducing intermediate filaments: from discovery to disease. *J. Clin. Invest.* 119, 1763–71. <https://doi.org/10.1172/JCI38339>
- Foeger, N., Wiesel, N., Lotsch, D., Mücke, N., Kreplak, L., Aebi, U., Gruenbaum, Y., Herrmann, H., 2006. Solubility properties and specific assembly pathways of the B-type lamin from *Caenorhabditis elegans*. *J. Struct. Biol.* 155, 340–350. <https://doi.org/10.1016/j.jsb.2006.03.026>
- Geisler, N., Schunemann, J., Weber, K., Haner, M., Aebi, U., 1998. Assembly and Architecture of Invertebrate Cytoplasmic Intermediate Filaments Reconcile Features of Vertebrate Cytoplasmic and Nuclear Lamin-type Intermediate Filaments. *J. Mol. Biol.* 282, 601–617. <https://doi.org/10.1006/jmbi.1998.1995>
- Gerace, L., Blum, A., Blobel, G., 1978. Immunocytochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction: Interphase and mitotic distribution. *J. Cell Biol.* 79, 546–566. <https://doi.org/10.1083/jcb.79.2.546>
- Goldman, R.D., Shumaker, D.K., Erdos, M.R., Eriksson, M., Goldman, A.E., Gordon, L.B., Gruenbaum, Y., Khuon, S., Mendez, M., Varga, R., Collins, F.S., 2004. Accumulation of mutant lamin A progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8963–8968. <https://doi.org/10.1073/pnas.0402943101>
- Gruber, M., Lupas, A.N., 2003. Historical review : Another 50th anniversary – new periodicities in coiled coils. *TRENDS Biochem. Sci.* 28, 679–685. <https://doi.org/10.1016/j.tibs.2003.10.008>
- Guzenko, D., Chernyatina, A.A., Strelkov, S. V., 2017. Fibrous Proteins: Structures and Mechanisms 82. <https://doi.org/10.1007/978-3-319-49674-0>
- Guzenko, D., Strelkov, S. V., 2018. CCFold: rapid and accurate prediction of coiled-coil structures and application to modelling intermediate filaments. *Bioinformatics* 34, 215–222. <https://doi.org/10.1093/bioinformatics/btx551>
- Heitlinger, E., Peter, M., Lustig, A., Nigg, E.A., 1991. Expression of Chicken Lamin B2 in *Escherichia coli*: Characterization of its Structure, Assembly, and Molecular Interactions. *Cell* 113, 485–495. <https://doi.org/10.1083/jcb.113.3.485>
- Heitlinger, E., Peter, M., Lustig, A., Villiger, W., Nigg, E.A., Aebi, U., 1992. The role of the head and tail domain in lamin structure and assembly: Analysis of bacterially expressed chicken Lamin A and truncated B2 lamins. *J. Struct. Biol.* 108, 74–91. [https://doi.org/10.1016/1047-8477\(92\)90009-Y](https://doi.org/10.1016/1047-8477(92)90009-Y)
- Herrmann, H., Aebi, U., 2004. Intermediate Filaments : Molecular Structure, Assembly Mechanism, and Integration Into Functionally Distinct Intracellular Scaffolds. *Annu. Rev. Biochem.* 73, 749–789. <https://doi.org/10.1146/annurev.biochem.73.011303.073823>
- Herrmann, H., Bär, H., Kreplak, L., Strelkov, S. V., Aebi, U., 2007. Intermediate filaments: From cell architecture to nanomechanics. *Nat. Rev. Mol. Cell Biol.* 8, 562–573. <https://doi.org/10.1038/nrm2197>
- Herrmann, H., Häner, M., Brettel, M., Müller, S.A., Goldie, K.N., Fedtke, B., Lustig, A., Franke, W.W., Aebi, U., 1996. Structure and assembly properties of the intermediate filament protein vimentin: The role of its head, rod and tail domains. *J. Mol. Biol.* 264, 933–953.

- <https://doi.org/10.1006/jmbi.1996.0688>
- Herrmann, H., Hesse, M., Reichenzeller, M., Aebi, U., Magin, T.M., 2002. Functional complexity of intermediate filament cytoskeletons: From structure to assembly to gene ablation. *Int. Rev. cell Mol. Biol.* [https://doi.org/10.1016/S0074-7696\(05\)23003-6](https://doi.org/10.1016/S0074-7696(05)23003-6)
- Hess, J.F., Budamagunta, M.S., Voss, J.C., FitzGerald, P.G., 2004. Structural Characterization of Human Vimentin Rod 1 and the Sequencing of Assembly Steps in Intermediate Filament Formation in Vitro Using Site-directed Spin Labeling and Electron Paramagnetic Resonance. *J. Biol. Chem.* 279, 44841–44846. <https://doi.org/10.1074/jbc.M406257200>
- Kabsch, W., 2010. XDS. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 66, 125–132. <https://doi.org/10.1107/S0907444909047337>
- Kapinos, L.E., Schumacher, J., Mücke, N., Machaidze, G., Burkhard, P., Aebi, U., Strelkov, S. V., Herrmann, H., 2010. Characterization of the Head-to-Tail Overlap Complexes Formed by Human Lamin A, B1 and B2 “Half-minilamin” Dimers. *J. Mol. Biol.* 396, 719–731. <https://doi.org/10.1016/j.jmb.2009.12.001>
- Kollmar, M., 2015. Polyphyly of nuclear lamin genes indicates an early eukaryotic origin of the metazoan-type intermediate filament proteins. *Sci. Rep.* 5, 1–10. <https://doi.org/10.1038/srep10652>
- Krissinel, E., Henrick, K., 2007. Inference of Macromolecular Assemblies from Crystalline State. *J. Mol. Biol.* 372, 774–797. <https://doi.org/10.1016/j.jmb.2007.05.022>
- Lupas, A., 1996. Coiled coils: New structures and new functions. *Trends Biochem. Sci.* 21, 375–382. [https://doi.org/10.1016/0968-0004\(96\)10052-9](https://doi.org/10.1016/0968-0004(96)10052-9)
- Makarov, A.A., Zou, J., Houston, D.R., Spanos, C., Cardenal-peralta, C., Rappsilber, J., Schirmer, E.C., Solovyova, A.S., 2019. Lamin A molecular compression and sliding as mechanisms behind nucleoskeleton elasticity. *Nat. Commun.* 10, 1–17. <https://doi.org/10.1038/s41467-019-11063-6>
- Mücke, N., Wedig, T., Bürer, A., Marekov, L.N., Steinert, P.M., Langowski, J., Aebi, U., Herrmann, H., 2004. Molecular and biophysical characterization of assembly-starter units of human vimentin. *J. Mol. Biol.* 340, 97–114. <https://doi.org/10.1016/j.jmb.2004.04.039>
- Omary, M.B., 2009. Review series introduction “IF-pathies”: a broad spectrum of intermediate filament – associated diseases. *J. Clin. Invest.* 119, 1756–1762. <https://doi.org/10.1172/JCI39894.1756>
- Östlund, C., Bonne, G., Schwartz, K., Worman, H.J., 2001. Properties of lamin A mutants found in Emery-Dreifuss muscular dystrophy, cardiomyopathy and Dunnigan-type partial lipodystrophy. *J. Cell Sci.* 114, 4435–4445.
- Pang, A.H., Obiero, J.M., Kulczyk, A.W., Sviripa, V.M., Tsodikov, O. V, 2018. A crystal structure of coil 1B of vimentin in the filamentous form provides a model of a high-order assembly of a vimentin filament. *FEBS J.* 285, 2888–2899. <https://doi.org/10.1111/febs.14585>
- Parry, D.A.D., 2006. Hendecad repeat in segment 2A and linker L2 of intermediate filament chains implies the possibility of a right-handed coiled-coil structure. *J. Struct. Biol.* 155, 370–374. <https://doi.org/10.1016/j.jsb.2006.03.017>
- Parry, D.A.D., Steinert, P.M., 1999. Intermediate filaments: Molecular architecture, assembly, dynamics and polymorphism. *Q. Rev. Biophys.* 32, 99–187. <https://doi.org/10.1017/S0033583500003516>
- Peter, A., Stick, R., 2015. Evolutionary aspects in intermediate filament proteins. *Curr. Opin. Cell Biol.* 32,

- 48–55. <https://doi.org/10.1016/j.ceb.2014.12.009>
- Schietke, R., Bröhl, D., Wedig, T., Mücke, N., Herrmann, H., Magin, T.M., 2006. Mutations in vimentin disrupt the cytoskeleton in fibroblasts and delay execution of apoptosis. *Eur. J. Cell Biol.* 85, 1–10. <https://doi.org/10.1016/j.ejcb.2005.09.019>
- Smith, T.A., Strelkov, S. V, Burkhard, P., Aebi, U., Parry, D.A.D., 2002. Sequence Comparisons of Intermediate Filament Chains : Evidence of a Unique Functional / Structural Role for Coiled-Coil Segment 1A and Linker L1. *J. Struct. Biol.* 145, 128–145. <https://doi.org/10.1006/jsbi.2002.4438>
- Steinert, P.M., Marekov, L.N., Fraser, R.D.B., Parry, D.A.D., 1993a. Keratin intermediate filament structure. Crosslinking studies yield quantitative information on molecular dimensions and mechanism of assembly. *J. Mol. Biol.* <https://doi.org/10.1006/jmbi.1993.1161>
- Steinert, P.M., Marekov, L.N., Parry, D. a D., 1993b. Diversity of intermediate filament structure. *J. Biol. Chem.* 268, 24916–24925.
- Strelkov, S. V., Burkhard, P., Mu, M.E., 2002a. Analysis of α -Helical Coiled Coils with the Program TWISTER Reveals a Structural Mechanism for Stutter Compensation. *J. Struct. Biol.* 137, 54–64. <https://doi.org/10.1006/jsbi.2002.4454>
- Strelkov, S. V., Herrmann, H., Geisler, N., Wedig, T., Zimbelmann, R., Aebi, U., Burkhard, P., 2002b. Conserved segments 1A and 2B of the intermediate filament dimer: Their atomic structures and role in filament assembly. *EMBO J.* 21, 1255–1266. <https://doi.org/10.1093/emboj/21.6.1255>
- Strelkov, S. V, Schumacher, J., Burkhard, P., Aebi, U., Herrmann, H., Mu, M.E., 2004. Crystal Structure of the Human Lamin A Coil 2B Dimer : Implications for the Head-to-tail Association of Nuclear Lamins. *J. Mol. Biol.* 343, 1067–1080. <https://doi.org/10.1016/j.jmb.2004.08.093>
- Stuurman, N., Heins, S., Aebi, U., 1998. Nuclear Lamins : Their Structure, Assembly, and Interactions. *J. Struct. Biol.* 66, 42–66. <https://doi.org/10.1006/jsbi.1998.3987>
- Testa, O.D., Moutevelis, E., Woolfson, D.N., 2009. CC+: A relational database of coiled-coil structures. *Nucleic Acids Res.* 37, 315–322. <https://doi.org/10.1093/nar/gkn675>
- The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC., n.d.
- Tickle, I.J., Flensburg, C., Keller, P., Paciorek, W., Sharff, A., Vornrhein, C., Bricogne, G., 2018. STARANISO. United Kingdom Glob. Phasing Ltd.
- Turgay, Y., Eibauer, M., Goldman, A.E., Shimi, T., Khayat, M., Medalia, O., 2017. The molecular architecture of lamins in somatic cells. *Nature* 543, 261–264. <https://doi.org/10.1016/j.bbi.2015.08.015>

Figure legends

Figure 1. Coil1b segment of the IF rod domain. A. Amino-acid sequence alignment of coil1b constructs used for structure determination, including the lamin A fragment (residues 65-222) discussed here, vimentin fragment (residues 144-251) (Aziz et al., 2012) as well as keratin K1 and K10 fragments forming a heterotetramer (Eldirany et al., 2019). The N- and C-terminal parts of coil1b with regular heptad repeats -common to all proteins are labelled in cyan. The 42-residue insert only present in lamins is in red. The untwisted region (parallel bundle, pb) at the C-terminus of coil1b is in green. The lamin construct additionally contains two last residues of coil1a (blue) and linker L1 (orange). The hydrophobic repeat pattern is given above the alignment, with the inserts shaded grey. Residues observed to effectively contribute to the coiled-coil core in the crystal structures are highlighted in yellow. Residues involved in the hydrophobic knob-into-hole interface contributing to the tetramer formation in vimentin and keratins (Eldirany et al., 2019) are in magenta and underlined. Asterisk (*) indicates fully conserved positions, colon (:) indicates highly similar residues and period (.) indicates weakly similar residues. B. Schematic representation of the lateral antiparallel association of lamin dimers yielding the A₁₁-type tetramer. 'Ig' stands for the globular immunoglobulin fold within the lamin tail domain.

Figure 2. Lamin coil1b dimer. A. Ribbon diagram of the AB dimer which is fully ordered in the crystal structure including the linker L1 (orange). The 42-residue insert is shown in red. The parallel bundle near the C-terminus is in green. B. Coiled-coil radius (blue line) and twist angle per residue (orange line) plotted against the residue number. C. Close-up on the linker L1 region and the beginning of coil1b. The hydrophobic core residues are shown as sticks.

Figure 3. Structural comparison of the lateral coil1b tetramers of lamin A (PDB code 6SNZ, this work), vimentin (PDB code 3UF1, Aziz et al., 2012), and keratin K1/K10 (PDB code 6ECO, Eldirany et al., 2019). A. Ribbon diagrams to scale. B. Superposition of the common structural motif (left-hand side) of the three tetramers. An arrowhead indicates the largest deviation within the distal part of the lamin tetramer compared to two others.

Figure 4. Main interactions responsible for the tetramer formation. A. The three tetramers shown to scale. Each time one dimer is shown as a molecular surface and the antiparallel dimer as a ribbon with selected side chains. Keratin K1 and K10 chains are drawn in grey and blue respectively. Linker 1 in lamin is shown in orange. Residues involved in the interdimer interface are indicated in bright green (polar residues) and yellow (apolar residues). Amino acids forming the knob/hole interaction are shown in magenta. B. A close-up on the unique central interface in the lamin tetramer. Grey lines indicate the α -helical axes as calculated by the program Twister (Strelkov et al., 2002a).

Tables

Table 1. Data collection and refinement statistics

Diffraction data

Number of crystals used	1
Space group	$P2_1$
Unit cell dimensions:	
a, b, c (Å)	88.07, 37.47, 101.68
β (°)	94.12
Resolution range (Å)	35.15-2.6 (2.7-2.6*)
$\langle I/\sigma \rangle$	8.62 (0.93)
CC $\frac{1}{2}$ (%)	0.999 (0.644)
Completeness (%)	97.0 (86.6)
No. of unique reflections	20594 (1810)
Redundancy	6.0 (4.1)
Wilson B-factor (Å ²)	50.2

Refinement

R _{work} (%)	25.4
R _{free} (%)	28.1
No. of protein chains per asymmetric unit	4
No. of non-hydrogen atoms:	
protein	4814
ligands/ions	12
water molecules	113
R.m.s. deviations:	
bonds (Å)	0.012
angles (°)	1.63
Ramachandran plot:	
favoured/allowed/outlier residues (%)	99.5/0.5/0
Side-chain rotamer outliers (%)	3.4
Clash score	1.22
PDB code	6SNZ

* Values in parentheses are for the highest resolution shell

Table 2. Intra- and interdimeric interfaces in lamin coil1b tetramer

Interface	Type	Area, Å ²	ΔG, kcal/mol	P-value	N _{HB}	N _{SB}
Intradimeric						
A/B	parallel	4007	-78.5	0.005	17	33
C/D	parallel	3834	-71.3	0.008	11	38
Interdimeric						
AB/CD	parallel	757	4.2	0.814	6	9
AB/C'D'	antiparallel	726	7.5	0.909	6	11
AB/A'B'	antiparallel	1612	-8.8	0.381	11	10
CD/C'D'	antiparallel	1422	-12.1	0.202	8	5

A' means a symmetry-related copy of chain A *etc.* The data were obtained using the program PISA (Krissinel and Henrick, 2007). ΔG is defined as the change in the solvation energy upon interface formation. This value thus does not include the contribution of hydrogen bonds or salt bridges. The P-value of the interface is defined as the probability to get a lower ΔG value than measured, by chance. For specific hydrophobic interfaces the P-value should be below 0.5. N_{HB}, number of hydrogen bonds. N_{SB}, number of salt bridges.

Table 3. Interdimeric coil1b interfaces in lamin, vimentin and keratin K1/K10 tetramers

Protein	Area, Å ²	ΔG, kcal/mol	P-value	N _{HB}	N _{SB}
Lamin total*	1574	-9.7	0.322	10	10
Lamin, central interface only	318	-3.5	0.597	4	0
Vimentin	1841	-8.7	0.325	20	20
Keratin	1982	-27.2	0.053	12	4

* Same interface as for AB/A'B' in Table 2 but with linker L1 excluded.

All values are defined as in Table 2.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Graphical abstract

Highlights

- A 2.6 Å crystal structure of lamin A coil1b segment is presented
- The dimers align laterally in antiparallel fashion driving the A₁₁ type assembly
- Common principles of the A₁₁ contact across intermediate filament types are revealed
- A lamin-specific sequence insert provides for in a 30% longer antiparallel interface
- Despite this, the contact appears weaker in lamins compared to cytoplasmic IFs