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cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins

(lamin sequence identity/divergent carboxyl termini/α-helical domains/coiled coils/nuclear localization sequence)

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ABSTRACT **The amino acid sequences deduced from cDNA clones of human lamin A and lamin C show identity between these two lamins except for an extra 9.0-kDa carboxyl-terminal tail that is present only in lamin A. Both lamins A and C contain an α-helical domain of approximately 360 residues that shows striking homology to a corresponding α-helical rod domain that is the structural hallmark of all intermediate filament proteins. However, the lamin α-helical domain is 14% larger than that of the intermediate filament proteins. In addition to the extensive homology to intermediate filament proteins as reported [McKeon, F., Kirschner, M. & Caput, D. (1986) *Nature (London)* 319, 463–468], a different 82-amino acid residue stretch at the carboxyl terminus of lamin A has been deduced and verified by amino acid sequencing. This region contains sequence homology to amino- and carboxyl-terminal domains of type I and type II epidermal keratins. Implications of the presence of these and other domains in lamins A and C for the assembly of the nuclear lamina are discussed.**

The nuclear lamina is a polymeric structure that is intercalated between chromatin and the inner membrane of the nuclear envelope. In mammalian cells the lamina consists primarily of three proteins (1–4) that have been termed lamins A (70 kDa), B (67 kDa), and C (60 kDa) (5). Peptide maps of lamins A and C are similar, but they are different from those of lamin B (6). However, certain monoclonal antibodies have been shown to crossreact with all three lamins, indicating that some epitopes are shared by all three lamins (7, 8).

The lamins have been shown to be synthesized throughout the cell cycle (9). *In vitro* translation of mRNA (10) showed that lamins A and C are synthesized from separate mRNAs with lamin A being made as a precursor approximately 2 kDa larger than mature lamin A. The precursor is converted into mature lamin A only after incorporation into the lamina structure, as shown by pulse–chase experiments (9).

The lamina is depolymerized during mitotic prophase by a process that may involve transient hyperphosphorylation (5). Concomitant with a gradual dephosphorylation, the lamins repolymerize in telophase (3, 5). How the regular protein meshwork (2) that constitutes the lamina is assembled from the three lamins is not known. The lamins must contain domains that are involved in this polymerization and that interact with chromatin on the nucleoplasmic face of the lamina, with the inner nuclear membrane on the other face of the lamina, and, most likely, with nuclear pore complexes (1, 2). As such domains could be revealed from the amino acid sequences of the lamins, we decided to clone their cDNAs. We report here the deduced amino acid sequences for lamins A and C. While this manuscript was in preparation, McKeon *et al.* (11) reported lamin sequence data that our findings corroborate and extend. A difference in predicted amino acid sequence of the carboxyl-terminal 82 residues of the lamin A precursor was noted and confirmed by amino acid sequencing. This carboxyl-terminal domain showed sequence homology to the head and tail domains of keratins, thereby extending lamin A sequence homologies with intermediate filament proteins to regions outside the α-helical rod domain. Furthermore, a 762-base-pair (bp) 3′-untranslated region including the polyadenylylation signal for lamin A is now reported.

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MATERIALS AND METHODS

Screening cDNA Libraries. Guinea pig antibodies to rat liver lamins A, B, and C (gift of L. Gerace) were used to screen a rat liver λgt11 cDNA library (gift of R. Hynes) according to established protocols (12, 13). Eight cDNA clones were obtained, the largest of which [1.3 kilobases (kb)] was used to screen (14) the simian virus 40-transformed human fibroblast cDNA library of Okayama and Berg (15) (gift of H. Okayama). A 2.6-kb partial cDNA clone of lamin A was obtained and sequenced (see below). A 22-bp oligonucleotide, TGGCCCCGCAGATCATGCAGCT, complementary to a 5′ portion of the 2.6-kb clone was synthesized (Applied Biosystems 380A DNA Synthesizer) and used to rescreen the fibroblast library. A 1.8-kb partial cDNA clone of lamin C was obtained. A 21-bp oligonucleotide, GCATCCCCGAGCTCGGCCTCG, complementary to a 5′ portion of the 1.8-kb clone was synthesized and used to screen (16) a human hepatoma λgt11 cDNA library (gift of M. Mueckler) for full length cDNA clones. A 1962-bp cDNA clone of lamin C was obtained.

RNA Blot Analysis. Nitrocellulose blots of HeLa cell poly(A)$^+$ RNA (prepared according to refs. 17–19) were hybridized with nick-translated rat liver cDNA or ^{32}P-labeled oligonucleotides. Nick-translated cDNA clones of the heat shock protein hsp70 (2.9 kb) and actin (2.0 kb) (gift of H. Kao) were hybridized as internal M_r markers. Blots were washed for 1 hr at 22°C, air dried, and exposed to Kodak XAR-5 film for 1–14 hr.

DNA Sequence Analysis. Restriction fragments of the plasmid and phage cDNA inserts were subcloned into M13 vectors (20) and sequenced in both orientations by the dideoxy chain termination method (21, 22). The rapid deletion subcloning procedure of Dale *et al.* (23) was used to sequence large fragments and to sequence across restriction sites. Maxam and Gilbert sequencing (24) was used to sequence DNA regions not adequately resolved by the dideoxy method.

Lamin Sequencing. Rat liver nuclear pore complex–lamina fraction was purified according to established procedures (2). Lamins were solubilized in 6 M urea/10 mM Tris·HCl, pH 8.0/20 mM dithiothreitol, loaded onto a DEAE-cellulose

Abbreviations: bp, base pair(s); kb, kilobase(s).

column equilibrated in the above buffer, and eluted with a 0–200 mM NaCl gradient (S. Georgatos and G.B., unpublished observations).

For acid hydrolysis of Asp-Pro bonds only, eluted fractions enriched in lamins A and C were electrophoresed on 7.5–15% $NaDodSO_4$/polyacrylamide gradient gels and electroblotted onto trifluoroacetic acid-treated glass fiber filters (Whatman GF/C) according to the low pH procedure of Aebersold *et al.* (25). Protein bands were visualized with Coomassie Blue, and lamin A and lamin C bands were cut from the filters. Asp-Pro cleavage was carried out by spotting 88% (vol/vol) formic acid (30 μl/cm^2 of filter strip) and incubating for 20 hr at 44°C in a sealed chamber saturated with 88% (vol/vol) formic acid (D. Atherton, personal communication). Filters were then air dried for 15 min.

For V8 protease digestion, fractions off the DEAE-cellulose column containing lamins A and C were pooled and precipitated with 10% (wt/vol) trichloroacetic acid; precipitates were resuspended in 0.2% $NaDodSO_4$/125 mM Tris·HCl, pH 6.8/20 mM dithiothreitol, and proteins were cleaved with V8 protease (Miles) as described (26). Samples were electrophoresed and electroblotted onto glass fiber filters as above. Protein bands were visualized with Coomassie Blue.

The lamin fragments on glass fiber filters were subjected to Edman degradation with the Applied Biosystems model 470A gas-phase sequencer. The phenylthiohydantoin amino acid derivatives were identified and quantitated using the Hewlett Packard 1084 HPLC system.

RESULTS

cDNA Cloning. A full-length lamin C clone (1962 bp) from a human hepatoma library and a partial lamin C clone (1.8 kb) and partial lamin A clone (2.6 kb) from a transformed human fibroblast library were obtained. The size of full-length lamin A and C cDNAs, 2.9 kb and 2.0 kb, respectively, was determined by RNA-blot analysis (Fig. 1).

DNA Sequence Analysis. Analysis of the two cloned cDNA sequences of lamin C (from both the fibroblast library and the hepatoma library) (Fig. 2) and the partial cDNA sequence of lamin A (from the fibroblast library) (Fig. 2) revealed complete identity in nucleotide sequences of lamins A and C with the exception of their 3′ ends. In addition, the 5′ end of

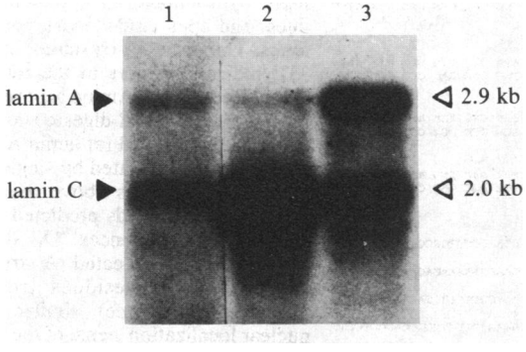

FIG. 1. Blot-hybridization analysis of HeLa cell poly(A)$^+$ RNA. A single lane of RNA (25 μg) was hybridized with ^{32}P-labeled nick-translated 1.3-kb rat liver cDNA alone (lane 1), with 1.3-kb rat liver cDNA, and then with actin cDNA (lane 2) or with 1.3-kb rat liver cDNA followed by actin cDNA and hsp70 cDNA (lane 3). The actin (2.0-kb) and hsp70 (2.9-kb) probes were used as internal molecular weight markers. Lamin A and C mRNA bands are seen in lane 1, the lamin A mRNA band is still visible in lane 2, but both lamin mRNAs are covered by the two molecular weight probes in lane 3. Similar results were obtained with ^{32}P-labeled oligonucleotide probes (data not shown).

full-length lamin A is identical to that of lamin C (11).

The predicted M_r of the encoded lamin C protein is 65,145, slightly larger than its apparent M_r of 60,000 as estimated by $NaDodSO_4$/PAGE (3) and in agreement with McKeon *et al.* (11).

The complete lamin A sequence (Fig. 2) has a predicted M_r of 74,152. This size is in close agreement with the apparent molecular size of 72 kDa of the lamin A precursor as estimated by $NaDodSO_4$/PAGE (9) and is about 5.3 kDa smaller than the size predicted by McKeon *et al.* (11).

Protein Purification and Sequencing. To confirm the identities of the cloned cDNAs, we attempted amino-terminal sequencing of purified rat liver lamin A and C polypeptides and found that both proteins were blocked. By V8 protease digestion we purified a 14-kDa fragment shared by both rat liver lamins. Fifteen amino acids sequenced from this rat liver fragment corresponded to residues 449–463 in human lamins A and C (Fig. 2).

Both lamins were also subjected to mild acid hydrolysis to cleave between Asp-Pro residues. Only one sequence (residues 477–486) was obtained from lamin C as predicted by the nucleotide sequence (Fig. 2). This same sequence was obtained from hydrolyzed lamin A; in addition, a second sequence was obtained from lamin A (residues 576–585) corresponding to the only other Asp-Pro site in the predicted lamin A sequence. The last three residues of this sequence (583–585) (Fig. 2) confirmed the predicted lamin A sequence where it diverged from that of McKeon *et al.* (11), who reported an additional nucleotide (cytosine) following codon 582.

These protein sequence data clearly established the identity of the two lamin clones.

Lamin Homology to Intermediate Filament Proteins. When the lamin A and C sequences were compared with other known protein sequences, a striking sequence homology was found with all types of intermediate filament proteins. In Fig. 3, the sequences of lamins A and C are compared to that of hamster vimentin (28) (serving as a representative of the family of intermediate filament proteins). An overlap of 366 residues between lamins A and C and of 318 residues of vimentin showed 27.6% identity.

Chou and Fasman analysis (30) to predict the secondary structure of lamins A and C revealed a 360-residue stretch of α-helical conformation extending from residue 31 to residue 390 (Fig. 2). Most strikingly, this region of predicted α-helical conformation coincides closely with the region of sequence homology between lamins A and C and vimentin (Fig. 3) and that of other intermediate filament proteins (29) (data not shown). An approximately 310-residue central domain of about 30% sequence homology and α-helical conformation is shared by all intermediate filament proteins (reviewed in ref. 31). The lamins contain a repeating heptad sequence within this α-helical domain of the form $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$ where residues a and d are usually hydrophobic residues (Fig. 2). Three regions within the α-helical domain, containing these heptad repeats, are separated by short stretches of amino acid residues that break up this repeating pattern. The α-helical domain of all intermediate filament proteins contains the same repeating heptad organization as the lamins. The three regions of heptad repeats are labeled Coil 1a, Coil 1b, and Coil 2 in Figs. 2 and 3, since such domains are thought to be capable of forming coiled coils with analogous domains of other molecules (32).

Fig. 3*a* shows alignment of these coiled coil forming regions between lamins A/C and vimentin with the exception of Coil 1b, which is about 50% longer in the lamins than in vimentin. Fig. 3*b* depicts this structural organization schematically.

The lamin A tail has a glycine- and serine-rich stretch of

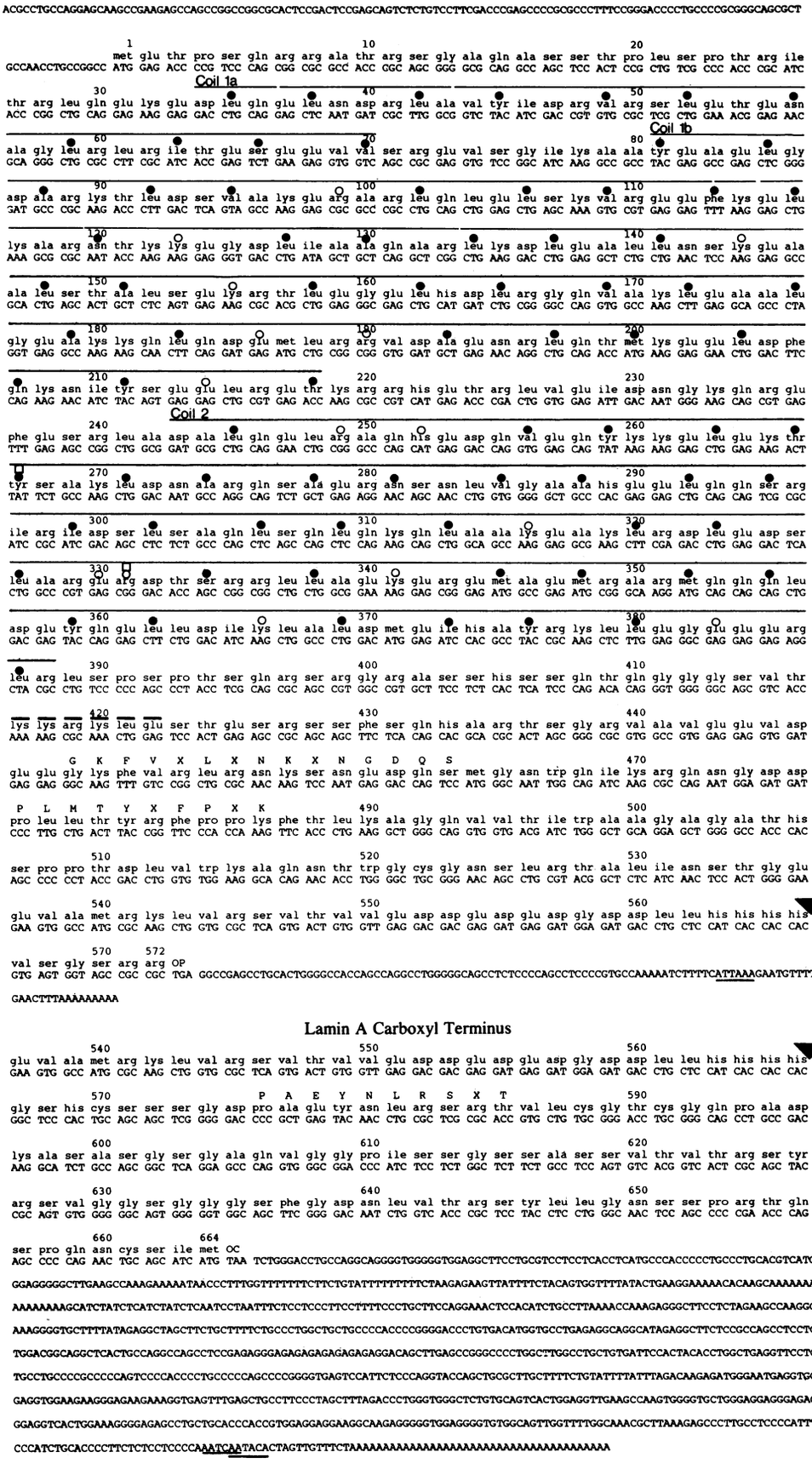

FIG. 2. cDNA sequences and deduced amino acid sequences of lamin C and lamin A. DNA sequencing was performed by the dideoxy chain-termination method on both strands of all cDNAs and by the chemical method on selected regions. Both of the isolated lamin C cDNA clones (1.8 kb and 1.9 kb) were sequenced as was the partial lamin A cDNA clone (2.6 kb). The 3'-polyadenylylation signals are underlined. Residue 150 is the first deduced amino acid of the incomplete lamin A cDNA sequence, and residue 566 (closed arrowhead) is the last amino acid shared by both lamins A and C before their sequences diverge. Coils 1a, 1b, and 2 denote α-helical domains with heptad repeats capable of forming coiled coils. At the first and fourth positions of the heptads, closed circles mark hydrophobic and nonpolar residues, and open circles mark polar residues. Open boxes (residues 267 and 331) indicate stutters in the repeating heptad sequences. Amino acid residues sequenced from V8-digested and formic acid-hydrolyzed rat lamin A and C fragments are indicated by single letter amino acid symbols above the corresponding amino acids predicted by the human cDNA sequences. "X" denotes amino acids not detected. A stretch of six amino acid residues (residues 417–422, broken line), similar to the nuclear localization signal of the simian virus 40 large T (tumor) antigen (27), is also indicated. Residues 551–560 at the carboxyl end of the lamin C protein (but within the carboxyl domain of the lamin A protein) are highly acidic.

residues (571–652) that shares approximately 20%–30% sequence homology with amino- and carboxyl-terminal portions of a human type II epidermal keratin (67 kDa) (33) (Fig. 4), with a mouse type II keratin (60 kDa) (36), and with mouse (59 kDa) and human (52 kDa) type I keratins (34, 35) (data not shown).

Nuclear Localization Site. Lamins A and C contain a sequence of 6 amino acids (Lys-Lys-Arg-Lys-Leu-Glu) that

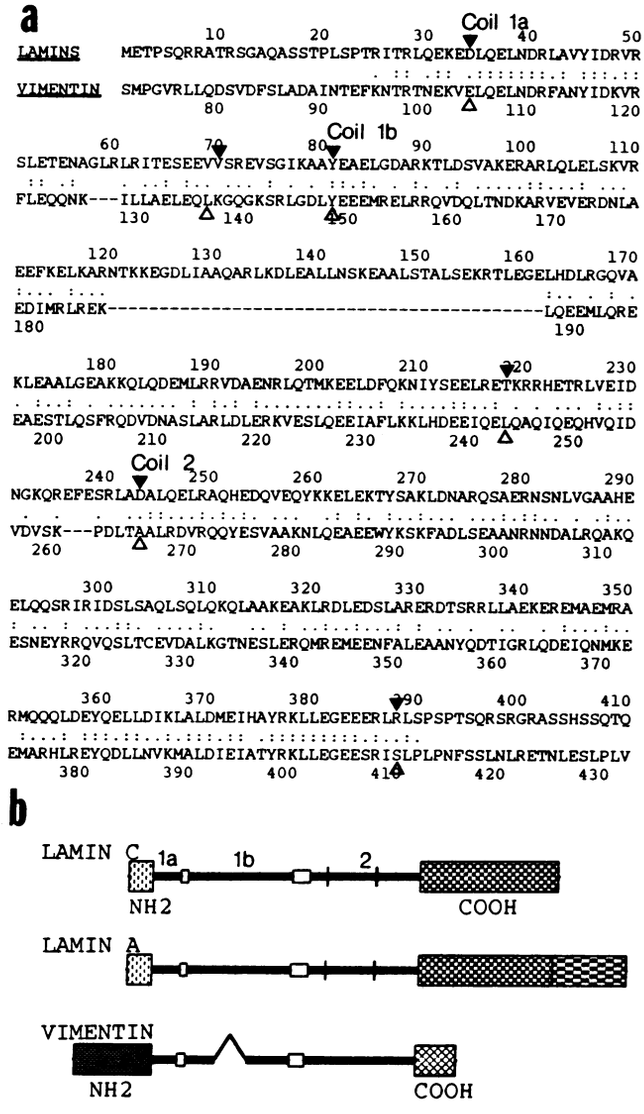

FIG. 3. Amino acid sequence homology and secondary structural homology between lamins A/C and vimentin. (*a*) The deduced amino acid sequence common to lamins A and C was aligned to maximize homology with that of hamster vimentin (28). The lamins shared 27.6% identity with vimentin over a 366-amino acid residue overlap. A gap of 42 residues was introduced into the vimentin sequence to optimize this homology. This region of sequence homology coincides with predicted α-helical domains capable of forming coiled coils present in lamins A/C (between closed arrowheads) and vimentin (between open arrowheads). The first 20 and last 35 amino acids within this stretch each show greater than 70% sequence homology. (*b*) Amino, carboxyl, and coiled coil domains of lamins A and C were aligned with the predicted secondary structure of hamster vimentin (29). All molecules are drawn to scale. Lamins A and C have identical sequences spanning the central α-helical domain (black lines) and the carboxyl domain of lamin C. The lamin C carboxyl domain extends 6 amino acids beyond the point of divergence from lamin A whereas the lamin A carboxyl domain extends 98 amino acids further (see Fig. 2). Putative globular domains are located at both ends of all three molecules. The central α-helical domains are 14% longer in lamins A/C than in vimentin. Coils 1a and 2 and the two intercoil linkers are conserved in size between the lamins and vimentin whereas Coil 1b is about 50% longer in the lamins than in vimentin. Vertical lines mark stutters in the heptad repeats.

is similar to a stretch of amino acids in the simian virus 40 large T (tumor) antigen (Lys-Lys-Arg-Lys-Val-Glu) that contributes to nuclear localization of the viral protein (27). This sequence is located approximately 30 amino acids outside the carboxyl end of the α-helical domain (Fig. 2).

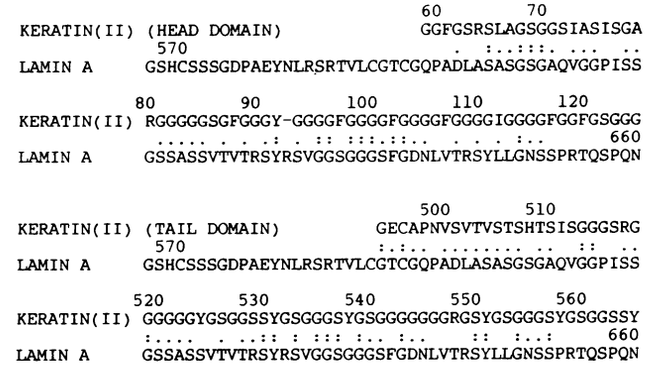

FIG. 4. Lamin A sequence homology to human type II (67 kDa) keratin head and tail domains. A 64-amino acid residue stretch (residues 589–652) in the carboxyl-tail region of lamin A, not present in lamin C (see Fig. 2), shows 20.3% and 26.6% identity with head and tail regions, respectively, of human type II (67 kDa) epidermal keratin (33). Similar homology is found to the head and tail regions of human type I (52 kDa) keratin (34) and mouse type I (59 kDa) and type II (60 kDa) keratins (35, 36) (data not shown).

DISCUSSION

Lamins A and C share an α-helical region of 360 amino acid residues that in turn shows striking homology with the canonical 310-residue α-helical rod domain characteristic of all intermediate filament proteins (Fig. 3) (11). Based on this sequence homology and the similar structural organization of the molecules (Fig. 3), lamins A and C belong to the family of intermediate filament proteins.

In spite of the structural relatedness between the lamins and the intermediate filament proteins, there are some noteworthy differences. The lamin α-helical domain is approximately 14% larger than the rod domain of intermediate filament proteins due to a longer Coil 1b domain (Fig. 3). Furthermore, in mitosis the lamins undergo complete and reversible depolymerization involving transient hyperphosphorylation whereas the intermediate filaments apparently do not. The large number of serine residues outside the α-helical domain and in the lamin A tail (Fig. 2) may be substrates for such kinase activity. Moreover, although both lamins and intermediate filament proteins belong to developmentally regulated gene families (reviewed in refs. 37 and 38), different forms of lamins appear to be expressed in nondividing versus dividing cells, whereas different intermediate filament proteins are expressed in cells depending on their embryonic origin.

Lamin A contains a 9.0-kDa carboxyl-terminal domain not present in lamin C (Fig. 2). McKeon *et al.* (11) have reported this domain to be 14 kDa. However, amino acid sequence data of rat lamin fragments have confirmed our predicted sequence in this region (Fig. 2). Furthermore, the lamin A cDNA contains a 977-bp 3′-untranslated region of which 762 bp were not present in the reported cDNA clone (11). Whether the precursor sequence of lamin A is part of the lamin A carboxyl-terminal domain is unknown. This domain contains many glycine and serine residues and shows homology to type I and type II keratins (Fig. 4 and data not shown). Unlike the keratins, however, this lamin stretch does not contain a tandemly repeating pattern of Gly-Gly-Gly-Xaa. The functional significance of this domain remains to be determined.

The lamin A cDNA sequence diverges from that of lamin C following a CAC codon corresponding to amino acid residue 566 (Fig. 2). Interestingly, the following six nucleotides in the lamin C sequence, GTGAGT, are identical to the consensus sequence of the 5′ end of introns (39). Also, the following two nucleotides in the lamin A sequence G-G represent a consensus sequence of the 5′ end of spliced exons

(39). In combination with evidence from Southern analysis that there may be only one gene coding for the two lamins (11), these sequence data suggest that the two lamins may arise from alternate forms of splicing. At the 3′ end of the gene transcript, the sequences containing the lamin C polyadenylylation site immediately downstream from codon 566 may be excised to splice a further downstream sequence to codon 566 to yield lamin A mRNA, or they may remain to yield lamin C mRNA.

The striking homology of lamins A and C with intermediate filament proteins suggests that at least some aspects of their assembly into a lamina may resemble that of intermediate filament proteins into intermediate filaments. Thus, coiled coil homodimers of lamins A and C may be formed by parallel interactions of their α-helical region. Such dimers might form rod-like structures with two smaller globular domains (comprising the amino terminus) at one end and two larger globular domains (comprising the carboxyl terminus) at the other end. Electron microscopy of dimers of the lamins from rat liver (U. Aebi, J. Cohn, L. Buhle, and L. Gerace, personal communication) shows that they contain two globular heads attached to an approximately 50-nm rod-like tail. Based on the sequence data, these globular head domains probably correspond to the carboxyl-terminal portions of the lamin polypeptides, which are larger than the amino-terminal portions.

How lamins A and C interact with lamin B to form a near-tetragonal lattice of 8- to 10-nm filaments (U. Aebi, J. Cohn, L. Buhle, and L. Gerace, personal communication) remains to be determined. As lamin B has a stronger physical association with the nuclear membrane (5), an additional domain present in lamin B may anchor the lamina to the inner nuclear membrane. However, it remains to be determined whether lamin B is also an intermediate filament-type protein.

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