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An Alternative Splicing Product of the Lamin A/C Gene Lacks Exon 10*

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Expression of the A-type lamins was studied in the lung adenocarcinoma cell line GLC-A1. A-type lamins, consisting of lamin A and C, are two products arising from the same gene by alternative splicing. Northern blotting showed in GLC-A1 a relatively low expression level of lamin C and an even lower expression level of lamin A as compared to other adenocarcinoma cell lines. Immunofluorescence studies revealed highly irregular nuclear inclusions of lamin A, suggesting protein or gene expression abnormalities. Reverse transcriptase-polymerase chain reaction-based cDNA analysis followed by sequencing indicated the presence of an as yet unidentified alternative splicing product of the lamin A/C gene. This product differs from lamin A by the absence of the 5′ part of exon 10 (90 nucleotides). Therefore we propose to designate this product lamin AΔ10. Deletion of the 30 amino acids encoded by exon 10 was predicted to result in a shift in pI of the protein from 7.4 to approximately 8.6, which was confirmed by two-dimensional immunoblotting. mRNA analysis in a variety of cell lines, normal colon tissue as well as carcinomas demonstrated the presence of lamin AΔ10 in all samples examined, suggesting its presence in a variety of cell types.

Lamins are intermediate filament-type proteins which form the major components of the nuclear lamina. Two main types of lamins are known in mammals, *i.e.* A-type lamins and B-type lamins. The B-type lamins, B1 and B2, are encoded by two distinct genes (1), while lamin B3 is a recently discovered alternative splicing product of the lamin B2 gene in embryonic cells (2). At least one of the B-type lamins is ubiquitously expressed in mammalian cells and their expression appears to be independent of the state of cellular differentiation (3, 4). The A-type lamins, represented by lamins A and C, are products arising from one gene by alternative splicing (5–7). A-type lamin expression appears to be related to the state of cellular differentiation. In general, well differentiated cells express A-type lamins, whereas undifferentiated cells synthesize low or undetectable levels of A-type lamins (8–10). In addition it is shown that A-type lamins are not expressed in proliferating cells of some adult tissues such as basal cells of the skin (11) or certain lineages of the hematopoietic system (12). The recent unraveling of the complete human lamin A/C gene (7) has provided a better insight into the mechanism by which lamins A and C are generated from the same gene. The site for alternative splicing has been demonstrated to be located within exon 10. While exon 1 through 9 of the lamin A and C mRNAs are identical, lamin A mRNA further contains the 5′ 90 bases of exon 10, followed by exon 11 and 12. In contrast the lamin C messenger contains the complete 111-base sequence of exon 10, but not exon 11 and 12. In this report we describe a third splicing product of the lamin A/C gene, identical to lamin A with the exception of the absence of exon 10.

EXPERIMENTAL PROCEDURES

Cell Lines—Adenocarcinoma cell lines NCI-H125, NCI-H23 (13, 14), GLC-A1, GLC-A2 (15), and NL-Ac1 (16), the neuroendocrine non-small cell lung carcinoma cell line NCI-H460 (17), the large cell undifferentiated lung carcinoma cell line LCLC-103H (18), the breast carcinoma cell line T47D (19), and the (bladder) transitional carcinoma cell line T24 (20) were grown in Roswell Park Memorial Institute Medium 1640 (RPMI 1640, ICN Flow, Irvine, UK) containing 2 g/liter $NaHCO_3$, supplemented with 10% newborn calf serum (ICN Flow). The epitheloid lung carcinoma cell line MR65 (21) was grown in minimal essential medium in 10% newborn calf serum, while the neuroblastoma cell line SK-N-SH (22) was grown in RPMI with 15% fetal bovine serum. All cell lines were maintained in a humidified incubator at 37 °C in 5% CO_2.

Human Tissue Specimens—Human lung adenocarcinoma specimens and a specimen of normal colon were obtained after surgery and snap frozen in liquid nitrogen. Samples were homogenized in ice-cold guanidine isothiocyanate buffer (23) using an Omni 1001 mixer (Omni, Waterbury, CT) at 20,000 rpm for 15–60 s and stored at −70 °C until use.

Immunofluorescence—The procedure for immunofluorescence microscopy has been described (24). Primary antibodies used include 133A2 (25) recognizing lamin A and LN43 (26) recognizing lamin B2. As a secondary antibody, fluorescein isothiocyanate-labeled rabbit anti-mouse immunoglobulins (DAKO, Glostrup, Denmark) was used.

RNA Isolation and Northern Blotting Analysis—Total RNA was isolated from cell lines and tissue specimens by the guanidine isothiocyanate procedure (23, 27). Twenty μg of total RNA were size-fractionated on a formaldehyde agarose gel (28), blotted onto Hybond-N+ membrane (Amersham Life Science, Little Chalfont, UK), and cross-linked with 120 J/cm^2 in an UV Stratalinker 1800 (Stratagene, La Jolla, CA). Blots were hybridized overnight at 65 °C in a hybridization mix containing 7% SDS (Life Technologies, Inc.), 1% bovine serum albumin (Sigma), 2 mM EDTA, 0.5 M Na_2HPO_4 (pH 7.0), and 0.1 mg/ml sheared salmon sperm DNA (Boehringer Mannheim GmbH, Mannheim, Germany), to which [^{32}P]dATP-labeled probe (see below) was added. After hybridization the blots were washed with saline citrate buffer (SSC, 0.15 M NaCl and 0.015 M sodium citrate pH 7.0) containing 0.1% SDS. Stringency conditions were optimized for each DNA probe. Autoradiography was performed with RX Fuji medical x-ray films (Fuji) at −70 °C using intensifying screens.

Probes—The lamin A/C probe, kindly provided by Dr. F McKeon (6), and the lamin B1 probe, a kind gift of Dr. Pollard (29), were labeled with [α-^{32}P]dATP (Amersham) by random priming (30) and purified over a Sephadex G50 fine (Pharmacia, Uppsala, Sweden) column. Each hybridization was performed with 50–200 ng of probe DNA. A GAPDH probe (31) was used as a control for the amount mRNA loaded onto the gel.

cDNA Synthesis and Polymerase Chain Reaction Analysis—The re-

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verse transcriptase (RT)[1] reaction was performed with 25 μg of total RNA or 5 μg of poly(A)$^+$ RNA. Samples were incubated for 1.5 h at 37 °C in a reaction mixture containing 10 μg of oligo(dT) primers (Pharmacia), 1 mM of each dNTP (Pharmacia), 40 units of RNasin (Promega, Madison, WI), 0.01 M dithiothreitol (Sigma), and 600 units of Moloney leukemia virus reverse transcriptase (Life Technologies, Inc.) in a total volume of 50 μl. After completion of the RT reaction 5 μl of this reaction mixture were used for PCR in a total reaction volume of 50 μl, containing 50 mM KCl (Merck), 10 mM Tris-HCl, pH 8.3 (Merck), 4 mM $MgCl_2$ (Merck), 0.001% gelatin, 1 mM of each dNTP (Pharmacia), 1.5 μg of primer 1 (sense, 5′-GCCTACCGCAAGCTCTTGGA-3′; Eurogentec, Seraing, Belgium), corresponding to lamin A codon 375–381 (nucleotides 1123–1142, see Fig. 2*A*), and 1.5 μg of primer 2 (antisense, 5′-GGTGAGGAGGACGCAGGAAG-3′, lamin A noncoding region, nucleotides 2030–2049). This mixture was heated to 95 °C for 5 min and cooled to 80 °C for 5 min, and thereafter 0.2 unit of *Taq* polymerase (Super *Taq*, HT Biotechnology Ltd., Cambridge, UK) was added. The mixture was overlaid with 60 μl of mineral oil (Sigma), heated at 94 °C for 2 min, and carried through 30 cycles of denaturation (94 °C, 1 min), annealing (54 or 58 °C, 2 min), and elongation (72 °C, 2 min), followed by a final extension step of 72 °C for 10 min. PCR products were used for subcloning (see below) or for a second round of PCR. For this latter purpose, samples were first run on 1.5% agarose gel. Bands at the 800–900-bp level, corresponding to the predicted size of products of lamin A and not lamin C cDNA, were excised and (electro-)eluted. Next, PCR was performed under identical conditions as described above, with primers 3 (sense, 5′-AGCCTGCGTACGGCTCTCAT-3′, lamin A codon 525–531, nucleotides 1573–1592) and 4 (antisense, 5′-GCTCCTGAGCCGCTGGCAGA-3′, lamin A codons 599–605, nucleotides 1795–1814).

Oligonucleotide Hybridization—PCR products were run on a 2% alkaline agarose gel (32), blotted onto Hybond N+ (Amersham) and hybridized with [γ-^{32}P]ATP end-labeled oligonucleotides. 5′-End labeling of 10 pmol of oligonucleotides was performed using the USB T4 polynucleotide kinase system (U. S. Biochemical Corp.). Oligonucleotides comprised primer 3 (see above), hybridizing to a region within exon 9, and an oligonucleotide complementary to the last 11 bases of exon 9 and the first 11 bases of exon 11 (oligo 5, 5′-CAGTGGGAGCCTTCCCCAGTGG-3′), thus enabling a specific hybridization to lamin A cDNA, in which exon 10 is lacking. Hybridization was performed at 46 °C in 5 × SSPE buffer containing 0.3% SDS. Washes were at 52 °C in the same buffer. The same blot was first hybridized with primer 3, stripped, and rehybridized with oligo 5.

Genomic DNA Analysis—DNA was isolated from human leukocytes, human placenta and cell lines GLC-A1 and NL-Ac1 using standard procedures (32). Purified genomic DNA was subjected to a single round of PCR using either primers 3 and 4 (see above) or primer 3 and primer 6, 5′-GAAAAGATTTTTGGCACGG-3′, complementary to the sequence in the untranslated region of exon 10. The use of primers 3 and 4 in PCR is supposed to produce a 1405-bp fragment for the lamin A/C gene, while primers 3 and 6 will yield a 649-bp fragment for this gene.

Plasmid Subcloning, Restriction Analysis, and Sequencing—Two products of 927 and 837 bp resulting from PCR with primers 1 and 2 were subcloned into the pGEM-T vector (Promega) and used to transform *Escherichia coli* TG1 cells by electroporation. Cloned plasmids were analyzed by restriction fragment analysis. The restriction digestions were performed with a combination of the endonucleases *Nco*I and *Sst*II, or *Sst*I and *Pst*I (Promega). Relevant restriction sites are indicated in Fig. 3*A*. Cloned fragments of the PCR reaction with primers 1 and 2 were digested with *Pst*I, and the fragment from nucleotide 1496–1707 was subcloned in pUC 19. Subsequently these fragments were sequenced using the dideoxynucleotide chain termination method (33) with 1.5–2 μg of template plasmid using a T7 sequencing kit (Pharmacia).

Two-dimensional Gel Electrophoresis and Immunoblotting—Cytoskeletal preparations of cell line GLC-A1 were prepared as described previously (24) and subjected to nonequilibrium pH gel electrophoresis (34). Electrophoresis was performed for 1800 V-h using 2% Bio-Lyte 3–10 carrier ampholytes (Bio-Rad). In the second dimension 10% SDS/polyacrylamide gels were used according to Laemmli (35). Proteins were immunoblotted onto nitrocellulose and the presence of lamin A was detected by antibody 133A2 (25) using enhanced chemiluminescence (ECL kit, Amersham, Buckinghamshire, UK) as described elsewhere (24).

[1] The abbreviations used are: RT, reverse transcriptase; PCR, polymerase chain reaction; bp, base pair(s).

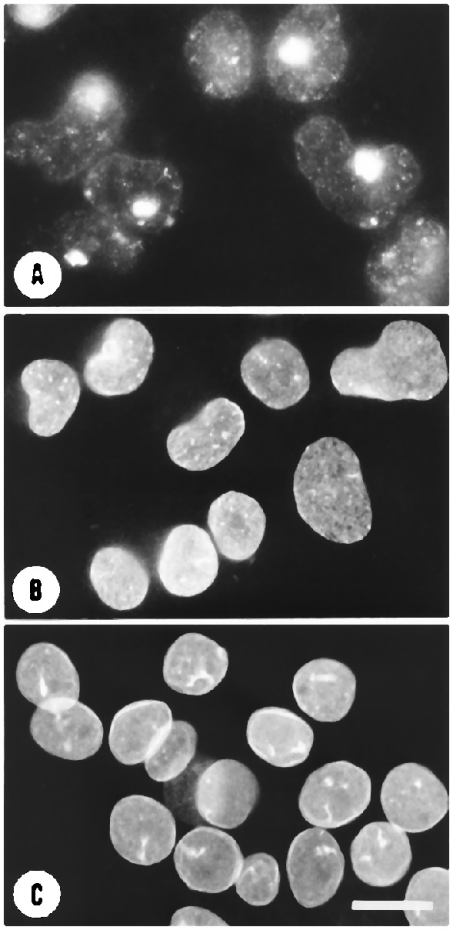


FIG. 1. **Immunofluorescence of cell line GLC-A1 (*Panels A* and *B*) and GLC-A2 (*C*), using an antibody to lamin A (*Panels A* and *C*) or lamin B2 (*Panel B*).** *Bar* represents 20 μm.

RESULTS

Immunofluorescence—In cell line GLC-A1 an abnormal localization of lamin A has been detected using immunofluorescence microscopy (Fig. 1*A*; see also Ref. 24). Instead of a perinuclear localization, as seen for the B-type lamins, nuclear aggregates of lamin A were seen in these cells, when stained with an antibody directed to lamin A. In contrast, lamin B2 appears to be organized in a perinuclear lamina (Fig. 1*B*) in the same cell line. Another adenocarcinoma cell line GLC-A2 expressed a normal fluorescence staining pattern with the lamin A antibody (Fig. 1*C*).

Northern Blotting—Northern blotting studies, comparing lamin expression in several adenocarcinoma cell lines of the lung, revealed that in the cell line GLC-A1 A-type lamin mRNA levels were largely reduced as compared to the other cell lines (Fig. 2). Lamin C mRNA levels in GLC-A1 were much lower than in the other cell lines and the lamin A messenger was barely detectable on Northern blot. No obvious abnormalities in the predicted sizes of the A-type lamin mRNAs were observed.

Detection of a New Type of Lamin A/C mRNA—To examine whether minor mRNA abnormalities could be detected, RT-PCR was performed. Special emphasis was put in the region coding for the carboxyl terminus since the structure of the lamin tail is crucial for correct nuclear assembly. Therefore, we designed primers 1 and 2 (Fig. 3*A*), starting at nucleotide 1123 and terminating 55 bases into the 3′-untranslated region of lamin A. Gel electrophoretic analysis of the PCR fragments of GLC-A1 showed two bands of about the predicted 927 bp (Fig.

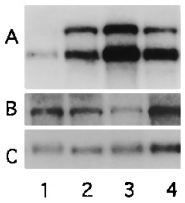


FIG. 2. **Northern blotting of cell lines GLC-A1, GLC-A2, NL-Ac1, and NCI-H125 (*lanes 1–4*), hybridized with the lamin A probe (*Panel A*).** Note the reduced expression of A-type lamins in GLC-A1 (*lane 1*). *Panel B*, lamin B1 probe; *Panel C*, GAPDH probe.

3*B*, *double arrows*). Only one band was found in a control PCR using a lamin A cDNA clone (not shown). In addition, RT-PCR was performed with primer 1 and primer 6, complementary to part of the 3′-untranslated region of lamin C mRNA, resulting in a single band around 650 bp, as predicted from the known cDNA sequence (data not shown). Subcloning of the two PCR products using primers 1 and 2 showed that one band was of the correct size (927 nucleotides), whereas the other product was approximately 100 bp shorter (Fig. 3*B*). Restriction fragment length analyses of the latter product showed that fragments containing exons 9, 10, and 11 were reduced by approximately 100 bp (Fig. 3*C*, *arrows*). Sequencing of both the normal and the short products revealed that in both products the nuclear localization signal and the CAA*X* motif were present (not shown), but that the 90 bp of exon 10 were missing in the short product. The predicted mRNA structure of this shortened lamin A, which we suggest to designate lamin AΔ10, is outlined in Fig. 3*D*. To map which region is missing from the normal lamin A, we have included a schematic drawing indicating the most important characteristics of lamin A (Fig. 3*E*). The amino acid region encoded by exon 10 is located in the carboxyl-terminal region, which forms the tail of the protein. This region contains a stretch of amino acids rich in aspartic acid and glutamic acid (Asp/Glu), followed by a sequence of four consecutive histidines (His_4).

Presence of Lamin AΔ10 in Other Cell Types—To determine whether lamin AΔ10 was unique for a single cell line, nested PCR was performed using primers 1 and 2, followed by PCR with primers 3 and 4 (Fig. 3*A*) on cDNA of several lung cancer cell lines, other carcinoma cell lines, and a neuroblastoma cell line. In addition, cDNA derived from tissues of normal colon and adenocarcinomas of the lung were examined (Fig. 4, *A* and *B*). PCR of plasmid lamin A cDNA yielded the expected 242-bp product of lamin A, whereas the lamin AΔ10 plasmid cDNA yielded a fragment of 152 bp (Fig. 4*A*, *lanes 1* and *2*). Analysis of cDNA from different sources on ethidium bromide stained agarose gels (*A* and *B*, *upper panels*) showed in addition to the expression of the 242 bp a band of 152 bp of variable intensity in most samples. The identity of the stained bands was confirmed by an alkaline agarose gel, and PCR products were hybridized to the end-labeled primer 3 (Fig. 4, *A* and *B*, *middle panels*) or oligo 5 (Fig. 4, *A* and *B*, *lower panels*). Hybridization to primer 3 showed that lamin A and lamin AΔ10 occur in all samples, except for the controls (*Panel A*, *lanes 1*, *2*, and *7*). However, the ratio of lamin A/AΔ10 expression showed a large variation among samples. Hybridization with oligo 5 allowed the positive identification of lamin AΔ10 in all samples tested, except for the two controls in *Panel A* (*lanes 2* and *7*). No detectable cross-hybridization with normal lamin A was observed.

Genomic DNA Analysis—To investigate the possibility that lamin AΔ10 is a transcript from an as yet unidentified lamin A-like gene, PCR was performed on genomic DNA samples from carcinoma cell lines and normal tissues. Fig. 5 shows that

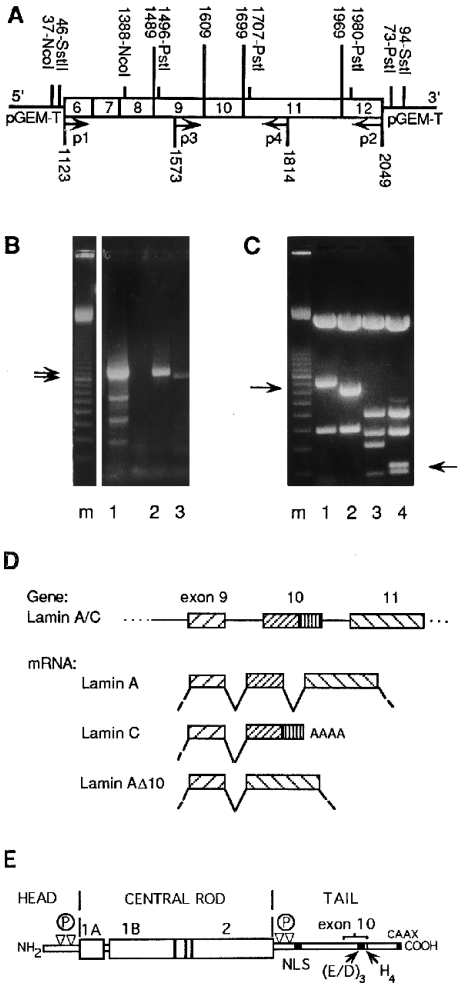
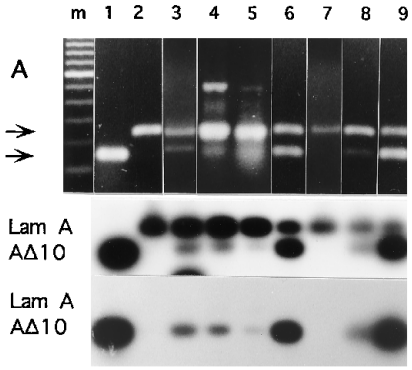


FIG. 3. *Panel A*, region of interest of the lamin A cDNA. Numbering started from nucleotide 1. *Upper numbers* indicate restriction sites and exon boundaries of interest. *Lower numbers* correspond to first nucleotide recognized by primers 1 through 4 (*p1–p4*). *Numbers in boxes* correspond to exon numbering according to Lin and Worman (7). *Panel B*, agarose gel electrophoretic analyses of PCR products resulting from primers 1 and 2 on cell line GLC-A1 (*lane 1*). Note the presence of two bands with sizes between 800 and 900 bp (*double arrow*). Excision and purification of each band, followed by electrophoresis, resulted in two bands with distinct molecular weights (*lanes 2* and *3*). *Panel C*, restriction fragment length analysis of the normal lamin A (*lanes 1* and *3*) and lamin AΔ10 (*lanes 2* and *4*) using *Sst*I and *Nco*I (*lanes 1* and *2*), or *Pst*I and *Sst*II (*lanes 3* and *4*). Note that after digestion with the first set of restriction enzymes the fragment of 704 bp (*lane 1*) is shortened in lamin AΔ10 (*lane 2*, *arrow*), and after digestion with the second set of restriction enzymes the expected fragment of 211 bp is shortened by approximately 100 bp (compare *lanes 3* and *4*, *arrow*). These results indicate a deletion between the *Pst*I sites of exon 9 and 11. *Panel D*, structure of the three different mRNAs resulting from alternative splicing of the lamin A/C gene. *Panel E*, schematic diagram (adapted from Nigg (42)) showing the impact of the missing exon 10 in lamin A at the protein level. *P*, phosphorylation site; *NLS*, nuclear localization signal; $(E/D)_3$, triple repeat of Glu and Asp; H_4, a sequence of four histidines; *CAAX*, motif for isoprenylation of lamin A.

DNA from placenta, leukocytes, and from cell lines GLC-A1 and NL-Ac1 all show a single band at the predicted levels of 649 and 1409 bp.

Two-dimensional Immunoblotting—Two-dimensional immunoblotting of GLC-A1 using the antibody to lamin A showed the presence of a lamin A doublet at the predicted position (approximately 70 kDa, pI 7.0, Fig. 6). In addition, however, a clear protein spot recognized by this antibody was observed with a molecular mass of around 65 kDa and a pI value that was shifted approximately 1 pH unit to the basic site (*arrow*). No



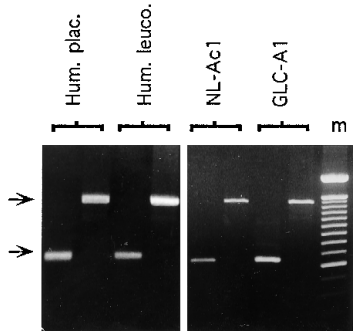


FIG. 5. **PCR-based analysis of genomic DNA of human placenta, human leukocytes, and cell lines NL-Ac1 and GLC-A1.** In the *first lane* for each sample, primers 3 and 6 were used, and in the *second lane* primers 3 and 4. *Arrows* indicate the level of the 1404-bp band (*upper*) and 649-bp fragment (*lower arrow*). Note that, in the 100-bp ladder markers (*m*), bands below 600 bp are barely visible.

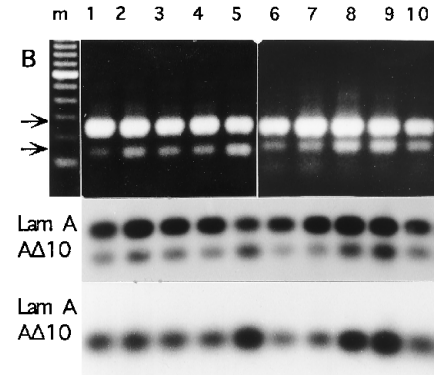


FIG. 4. **Analysis of PCR products for the presence of lamin A and lamin AΔ10 cDNA.** PCR using primers 1 and 2 was followed by a PCR using primers 3 and 4 in all cases, except for cell lines LCLC-103H and NCI-H125, which were subjected to a single round of PCR reactions using primer 3 and 4. *Panel A*, lamin AΔ10 cDNA (*lane 1*), normal lamin A cDNA (*lane 2*), GLC-A1 (*lane 3*), LCLC-103 (*lane 4*), NCI-H125 (*lane 5*), NCI-H23 (*lane 6*), normal lamin A cDNA (*lane 7*), GLC-A2 (*lane 8*), and NL-Ac1 (*lane 9*). *Panel B*, cDNA from cell line T24 (*lane 1*), T47D (*lane 2*), NCI-H460 (*lane 3*), MR65 (*lane 4*), SK-N-SH (*lane 5*), normal colon (*lane 6*), and four different adenocarcinomas of the lung (*lanes 7–10*). The *upper panel* shows ethidium bromide-stained agarose gels with levels of the expected lamin A and lamin AΔ10 bands denoted by an *arrow*. *Middle panel*, hybridization with ^{32}P-end-labeled primer 3, hybridizing to both lamin A and lamin AΔ10 cDNA. *Lower panel*, hybridization with ^{32}P-end-labeled oligonucleotide 5, specifically hybridizing to lamin AΔ10 cDNA only. *m* = 100-bp ladder markers

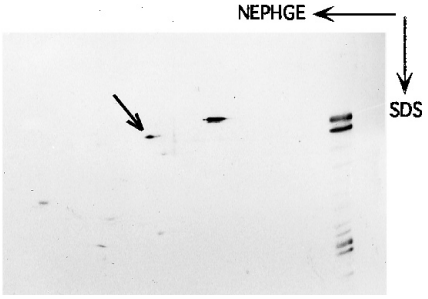


FIG. 6. **Immunoblot detection of lamin A and lamin AΔ10 after two-dimensional nonequilibrium gel electrophoresis.** Note the presence of normal sized lamin A with the expected pI of around 7.0, while an additional spot with lower molecular weight and more basic pI is also found (*arrow*).

such protein spot was observed in immunoblots of cell line NCI-H125 (not shown).

DISCUSSION

In this report we describe the widespread occurrence of an as yet unidentified splicing product of the lamin A/C gene that we designate lamin AΔ10, since exon 10 is absent in this transcript. In a previous report we have demonstrated the presence of an A-type lamin protein, which forms intranuclear aggregates in cell line GLC-A1 (24). In contrast to normal, perinuclear A-type lamins, these intranuclear aggregates could be largely extracted by Triton X-100, indicating that these aggregates are not assembled into the nuclear matrix (36). This abnormal nuclear localization of the protein suggested a distortion in the mRNA region coding for the carboxyl-terminal part of the protein, since this part is known to govern targeting to the nucleus by the nuclear localization signal, and the CAA*X*-motif, the isoprenylation site of (pre)lamin A, which is essential for a proper incorporation into the nuclear lamina (37–39). The structure of this region of the mRNA was examined by RT-PCR. Sequencing showed that the correct sequence for both motifs was present in the cDNA examined. However, gel analysis showed the presence of an additional shortened PCR product. Restriction fragment analysis, followed by sequencing showed that in the otherwise normal lamin A cDNA sequence exon 10 was lacking. Therefore we designated this novel protein lamin AΔ10.

Hybridization with oligonucleotides hybridizing to either lamin AΔ10 cDNA alone or both lamin AΔ10 and lamin A cDNA showed that lamin AΔ10 is expressed in a variety of tissues, since all samples examined were positive. In addition, we found that the ratio of expression levels of lamin AΔ10 and lamin A varied significantly between samples. Especially in the lung cancer cell lines the relative concentration of the lamin AΔ10 can be high. Similar differences in the expression ratio were observed between lamin A and lamin C at both protein and mRNA level (8, 24). Which mechanism is involved in regulating the relative expression of the three alternative splicing products of the lamin A/C gene, remains to be elucidated. Preliminary studies show that different cultures from the same cell line can express different ratios of lamin A to lamin AΔ10, which might be explained by differences in cell density.

A positive identification of the protein encoded by the lamin AΔ10 mRNA is not yet possible since no antibody specific for this product is available. Therefore we cannot yet state that the abnormal lamin A expression patterns as seen in GLC-A1 are represented by the lamin AΔ10 protein. However, evidence that indeed lamin AΔ10 mRNA is translated into protein comes from one-dimensional (24) and two-dimensional gel electrophoresis followed by immunoblotting studies. Computer assisted calculation of the pI of the lamin A Δ10 protein indicates

a value of 8.58 as compared to a theoretical pI of 7.4 for normal lamin A (PepStats, CAOS/CAMM, Nijmegen, The Netherlands). Furthermore, a deletion of 30 amino acids should give rise to an approximately 3.5-kDa smaller protein. In one-dimensional gel electrophoresis a protein smaller than lamin A was detected with the lamin A antibody 133A2 (24, 25). The additional protein spot detected in two-dimensional immunoblotting is significantly more basic (approximately 1 pI value), about 5 kDa smaller than the normal lamin, and fulfils the predicted electrophoretic characteristics of lamin AΔ10. The possibility that lamin AΔ10 is a result of a translocation or deletion of lamina A/C or is a transcript from a closely related as yet unknown gene has been examined. A previous study suggests that such a gene might exist (40). Our PCR analysis within the region between exon 9 and 11 of genomic DNA provides no evidence for an additional gene. Thus, a single gene is responsible for lamins A, AΔ10, and C. The possibility that lamin AΔ10 is the result of a mutated lamin A/C gene has been eliminated by our finding that lamin AΔ10 occurred in all samples examined.

It is feasible that the presence of lamin AΔ10 mRNA has been overlooked in previous studies because of its relatively low abundance as compared to lamins A and C expression. A cell line with a low expression of normal lamins A and C has enabled us to identify this lamin AΔ10 mRNA by RT-PCR. The same holds true for protein analyses, in which lamin AΔ10 is easily overlooked, especially because of the relatively large pI shift. Furthermore, only in cells expressing low levels of the normal A-type lamins an aberrant lamin can induce visible effects on the structure of the lamina. Apparently, in GLC-A1 the concentration of lamin AΔ10 can reach relatively high levels resulting in a distorted nuclear phenotype. The lamin AΔ10 protein may be localized in the nuclear inclusions seen in this cell line (24). This would be in agreement with transfection studies (39) which showed that different types of nuclear distortions can be induced with constructs of lamin A containing carboxyl-terminal deletions either starting at codon 456 (within exon 7) or starting at codon 550 (within exon 10). An altered localization of the lamin AΔ10 protein is likely, since the deletion of the first part of exon 10 results in loss of an acidic domain (7 consecutive Glu or Asp residues) and a polyhistidine domain. This is bound to have an impact on the interaction of lamin AΔ10 with other nuclear components. Although suggested, it is not yet shown that this particular highly charged region is involved in chromatin binding (41). If the protein extracted by Triton X-100 from GLC-A1 indeed represents lamin AΔ10, then it is possible that this protein is not or only partially bound to the nuclear matrix and may be involved in other intranuclear interactions.

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