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# Lamins of Ocular Lens Epithelial Cells

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**Abstract** Experiments were performed to characterize a prominent nuclear matrix (NM) protein isolated from tissue cultured mouse lens epithelial cells. This NM protein was separated by SDS-PAGE and the stained gel band was analyzed by mass spectroscopy. Blast analysis of the amino acid sequence derived by mass spectroscopy revealed the presence of Lamin C in the NM of the mouse lens epithelial cells. We also examined nuclear proteins of adult and fetal human lenses. Data collected from these experiments showed the presence of Lamin C in both adult and fetal lens cells. However fetal lens cells only show Lamin C dimers, whereas adult human lens contained dimers, monomers and degraded Lamin C. Early and late passaged tissue cultured mouse lens epithelial cells also contained Lamin C in the nucleus with a preponderance of the dimer in the early passaged cells. The biological significance of the presence of dimers in human fetal lens cells and early passaged mouse lens cells is not known. However, it could suggest an enhanced docking capability of Lamin C dimers for other physiologically important nuclear proteins. J. Cell. Biochem. 100: 923–928, 2007. © 2006 Wiley-Liss, Inc.

**Key words:** Lamin C; nuclear matrix; lens epithelial cells; adult and fetal human lens; nucleoplasm

The nuclear matrix (NM) is a morphologically complex network of anastomosing ribonucleoprotein strands that participates in the three dimensional organization of the nucleus [Fey et al., 1986]. Stein et al. [1996] reported that the NM participates in the discrete localization of specific genes, thus facilitating interaction with transcription factors. Previously, we reported presence of heat shock transcription (HSF-1) in the NM of mouse lens epithelial cells [Bagchi et al., 2001]. In this investigation, we isolated and characterized another NM protein with similar molecular weight. Mass spectroscopic analysis of this protein band revealed the presence of Lamin C in the NM of the αTN-4 cells. Moir et al. [1995] reported that lamins are prominent NM protein present in most eukaryotic cells. The nuclear lamins are classified as type V intermediate size filaments. Lamins A and C are somatic lamins that are identical in amino-acid sequence except that Lamin C has a unique six amino acid extension and lacks the CaaX target sequence. The Lamins A/C are splice variant of Lamin A (LMNA) gene [Gruenbaum et al., 2000].

Nuclear lamins are major constituents of the nuclear organization and play a role in the control of gene expression [Goldman et al., 2002]. Mutations in Lamin A have been linked to many human diseases such as Hutchinson–Gilford progeria syndrome (HGPS), atypical Werner's syndrome etc. [Raharjo et al., 2001; Goldman et al., 2005; Schirmer and Gerace, 2004]. Patients with atypical Werner's syndrome show a high incidence of early onset cataract, atherosclerosis, and diabetes [Mounkes and Stewart, 2004].

Many biologically important proteins including transcription factors and signaling proteins are known to bind to Lamin C, and probably any alteration of those bindings could result in impaired gene expression [Moir et al., 1995; Frock et al., 2006]. Here we report the presence of Lamin C in adult and fetal human lens cells, and in early and late passaged mouse lens epithelial cells.

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## METHODS

### Isolation of Nuclear Matrix Proteins

The NM proteins were extracted by the modified Penman technique [Bidwell et al., 1993] from confluent transgenic mouse lens epithelial cells (αTN-4) [Yamada et al., 1990] grown in tissue culture. The αTN-4 cells are epithelial cells obtained from transgenic mice. The mice were made transgenic by insertion of a hybrid gene comprising the murine $\alpha_{A\ promoter(-366-+46)}$, fused to the coding sequence of the SV-40 T antigen. The αTN-4 cells are widely used for lens research and retain tissue specific property of crystalline synthesis, a specific marker of ocular lens cells [Yamada et al., 1990]. Human adult and fetal lenses were obtained from the Wayne State University, School of Medicine, and Body Bequest Program. The matrix proteins, were extracted in a series of buffers containing detergent and nucleases to isolate NM intermediate filament (IF) Scaffold [Bagchi et al., 2001]. The NM-IF Scaffold was dissolved in disassembly buffer (8M urea, 20 mM MES, 1 mM EGTA, 12 mM PMSF, 0.1 M $mgCl_2$, 1% BME of pH 6.6) and than dialyzed against assembly buffer 0.15 M KCI, 2.5 mM imidazole hydrochloride, 2 mM DTT, 0.125 mM EGTA, 0.2 mM, 5 mM $mgCl_2$, PMSF of pH 7.1 overnight at room temperature. The dialysate was centrifuged at 100,000*g* for 95 min to separate soluble NM proteins from the pellets of intermediate sized filaments. The soluble NM proteins were concentrated using a centricon concentrator. The amount of protein in the soluble NM was determined by Bradford analysis [Bidwell et al., 1993]. Human lens epithelial cells were removed from the intact lens and isolated in hypotonic buffer and extracted nuclei were dissolved in 2% SDS-buffer. All proteins were separated by 10% polyacrylamide gels containing 0.1% SDS. SDS-PAGE gels were stained with Coomassie blue.

### Immunoblot

Proteins were transferred from polyacrylamide gels onto nitrocellulose paper according to Towbin et al [Bagchi et al., 2001]. Immunoblots were developed as previously described [Bagchi et al., 2001] with secondary antibody conjugated to alkaline phosphatase. Antibodies to Lamin C were purchased from Stressgen, Canada.

### Amino Acid Sequence Determination

The αTN-4 NM proteins were separated by 10% SDS-PAGE, and then stained with Coomassie blue. The stained 60 kDa protein doublet was cut from the gel and the amino acid sequence of the peptide was obtained by MALDI-TOF [Zhang et al., 1998]. The Coomassie stained SDS-PAGE gel piece containing 20 ng or more peptide was destained with Biorad destaining solution and then exposed to a methanol acetic acid solution (1:1) for 4 h at room temperature. The destained gel was further washed in distilled water. The distained gel containing peptide of interest was macerated and dissolved in a trypsin containing solution for 90 min at 37°C. The peptide was extracted with acetonitrile. Two steps mass spectrometry (MS/MS) was used to sequence digested peptide. Amino acid sequence obtained by MALD-TOF was then compared to the known sequences of the protein data base [Mann et al., 2001].

### Immuno Fluorescence Studies

The αTN-4 cells from early and late passaged cells were grown on cover slips. The cells were washed with cold phosphate buffered saline (PBS) than fixed in 4% paraformaldehyde on ice for 20 min. The fixed cells were incubated with rabbit anti-lamin C IgGs at 37°C for 1 h. Cells were then washed with PBS, and exposed to goat anti-rabbit IgG fluorescein conjugated antibody (1:500) at 37°C for 30 min. The cells were thoroughly washed with PBS and viewed under a fluorescence microscope.

## RESULTS

The NM proteins of tissue cultured lens epithelial cells were isolated by the modified Penman technique [Bidwell et al., 1993]. Figure 1 displays the protein profile of the total soluble NM fraction. A prominent doublet is present at 60 kDa. This is an interesting region as previously we isolated a NM protein (HSF-1) of similar molecular weight by non-equilibrium 2-D gel electrophoresis. To further identify this protein doublet, we determined the amino-acid sequence by mass spectroscopy. Figure 2 shows the amino acid sequence of the 60 kDa doublet. The NCBI blast search showed that this protein has over 99% sequence similarities with mouse Lamin C and C2.

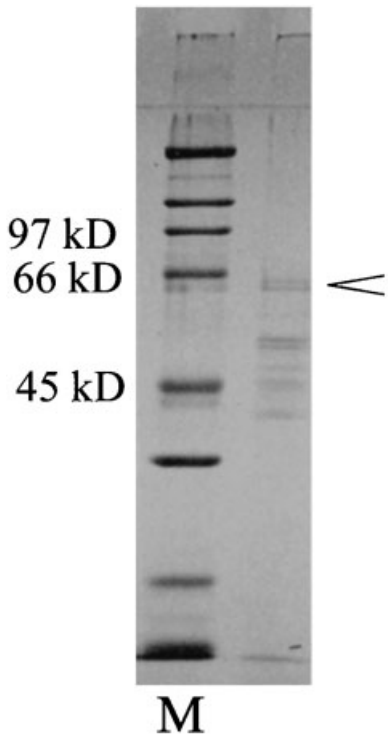


**Fig. 1.** SDS-PAGE profile of nuclear matrix component of mouse lens epithelial cells. M = molecular weight markers. Arrow points to a prominent peptide doublet.

Once we established the presence of Lamin C in the NM of tissue cultured mouse lens epithelial cells, we examined the NM of adult and embryonic human lenses for the presence of Lamin C. Figure 3 shows the immunoblot of nuclear proteins isolated from epithelial cells of adult white male (62 years) human lenses. Lamin C antibodies detected protein at 50, 60, and 120 kDa. The Lamin antibody interaction at 50 kDa may be result of Lamin C breakdown. The immuno reaction at 120 kDa reflects the presence of a Lamin C diamer. Figure 4 shows the nuclear protein profile of embryonic human lens epithelial cells of 20, 21 and 35 weeks old fetuses. It is interesting to note that Lamin C is present as a dimmer in all embryonic lenses and there was no breakdown product of Lamin C. Figure 5 shows the immunoblot of total proteins isolated from early and late passaged mouse lens epithelial cells. Lamin-c antibodies detected protein at 60 kDa.

Figure 6 shows the immunofluorescent profiles of tissue cultured mouse lenses. We examined tissue cultured cells from early and late passaged αTN-4 cells. The figure clearly show a punctuate presence of fluorescein, and both early and late passaged cells displayed similar patterns.

## DISCUSSION

This study was initiated to investigate the NM associated proteins of human and tissue cultured mouse lens epithelial cells. The SDS-PAGE profile of the NM fraction revealed a prominent doublet in the 60 kDa region Amino acid sequence of this doublet obtained by mass spectroscopy was compared with known amino acid sequences using the Blast technique [Altschul et al., 1990]. Analysis revealed that the stained band of NM fraction contain Lamin C and C2. This result was confirmed by immunoblots. Lamin C is developmentally regulated and primarily expressed in differentiated cells [Goldman et al., 2002] however epithelial cells of both adult and fetal lenses are not regarded as differential cells and presence of Lamin C as dimer in the fetal lens cells were not reported earlier. It is also interesting to note that both tissue cultured and human lens epithelial cells contain significant amounts of Lamin C.

Our experiments revealed that Lamin C is present in both adult and fetal human lenses.

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  1 METPSQRRAT RSGAQASSTP LSPTRITRLQ EKEDLQELND RLAVYIDRVR
 51 SLETENAGLR LRITESEEVV SREVSGIKAA YEAELGDARK TLDSVAKERA
101 RLQLELSKVR EEFKELKARN TKKEGDLLAA QARLKDLEAL LNSKEAALST
151 ALSEKRTLEG ELHDLRGQVA KLEAALGEAK KQLQDEMLRR VDAENRLQTL
201 KEELDFQKNI YSEELRETKR RHETRLVEID NGKQREFESR LADALQELRA
251 QHEDQVEQYK KELEKTYSAK LDNARQSAER NSNLVGAAHE ELQQSRIRID
301 SLSAQLSQLQ KQLAAKEAKL RDLEDSLARE RDTSRRLLAE KEREMAEMRA
351 RMQQQLDEYQ ELLDIKLALD MEIHAYRKLL EGEEERLRLS PSPTSQRSRG
401 RASSHSSQSQ GGGSVTKKRK LESSESRSSF SQHARTSGRV AVEEVDEEGK
451 FVRLRNKSNE DQSMGNWQIR RQNGDDPLMT YRFPPKFTLK AGQVVTIWAS
501 GAGATHSPPT DLVWKAQNTW GCGSSLRTAL INSTGEEVAM RKLVRSLTMV
551 EDNEDDDEDG EELLHHHRVS GSRR
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**Fig. 2.** Amino acid sequence of nuclear matrix peptide shown in Figure 1 (see arrow). The amino acid sequence was obtained by mass spectroscopy.

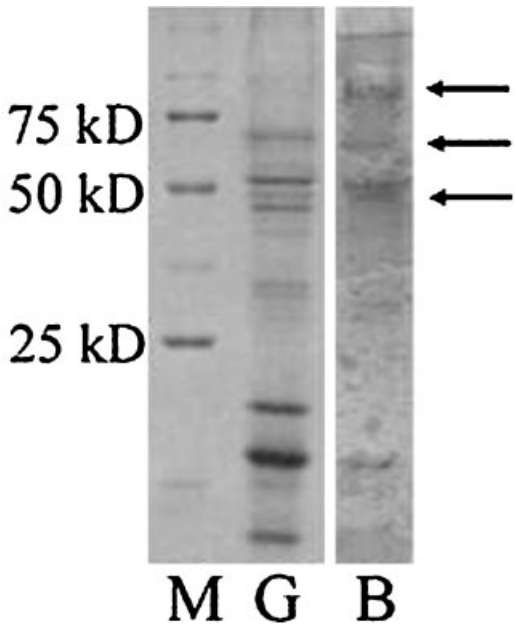


**Fig. 3.** Immunoblot of adult (62-year-old male) human lens epithelial cell protein with antibodies against Lamin C. M = markers; G = SDS-PAGE profile of lens epithelial cell protein; B = immunoblot with anti Lamin C.

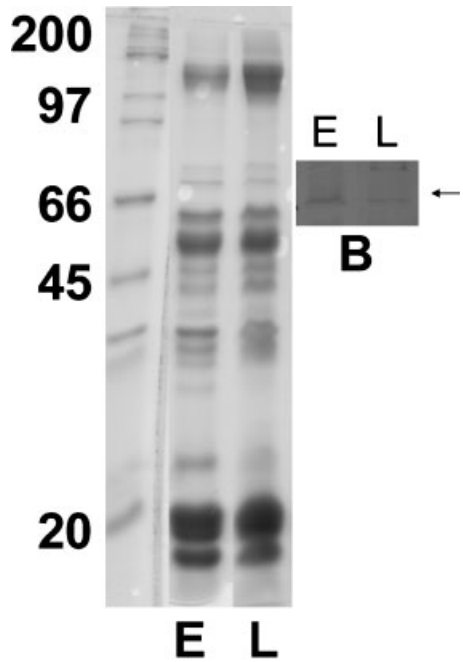


**Fig. 5.** Immunoblot of early and late passaged mouse lens epithelial cell total nuclear protein E and L = early and late passaged epithelial cells B = Immunoblot with anti-lamin C.

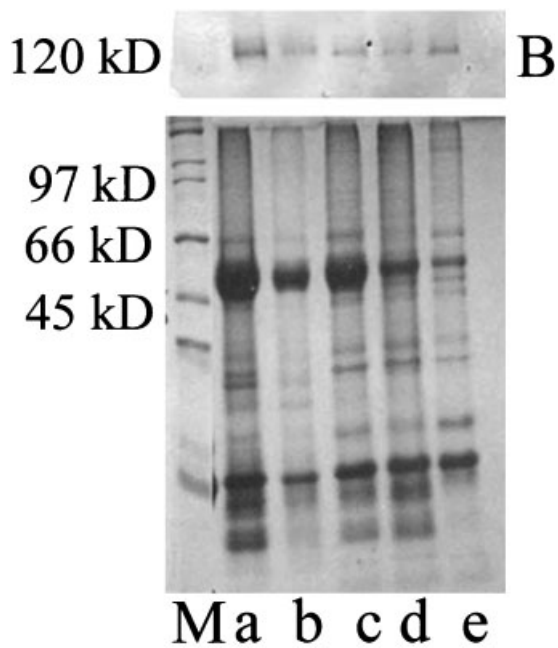


**Fig. 4.** Immunoblot of embryonic human lens epithelial cell proteins with anti-Lamin C antibodies. M = marker; B = Immunoblot. A–E. SDS-PAGE of lens epithelial cell proteins obtained from fatal human lens. A = 21 weeks, B = 20 weeks, C = 21 weeks, D = 20 weeks, E = 35 weeks.

We used fetal lenses obtained from 20 to 35 weeks of gestation. It is interesting to note that in all five fetal lenses, Lamin C is present as a dimer (120 kDa), whereas Lamin C in the adult human lens is found as both monomer and dimer, and a lower molecular weight band (50 kDa) indicating possible denaturation of Lamin C in the adult lens. It has been reported that the majority of nuclear proteins bind to Lamin C dimers and not polymers [Zastrow et al., 2004]. Furthermore Lamin C's are known to form a Variety of distinct oligomers that have specific docking properties for transcription factors [Zastrow et al., 2004], and any alteration in the integrity of Lamin C can change the docking properties of essential nuclear proteins resulting in depressed gene expression [Eggert et al., 1991]. Pathophysiology associated with the function of Lamin C may be caused by (a) failure to provide attachment to transcription factors or (b) reduced binding affinity to essential protein [Zastrow et al., 2004]. The functional role of Lamin C in either the maintenance of nuclear organization or specific gene expression of lens epithelial cells is not known. It has been suggested that repeated passaging of tissue cultured cells induce cell aging. Scaffidi and Misreli [2005] reported that cell lines from

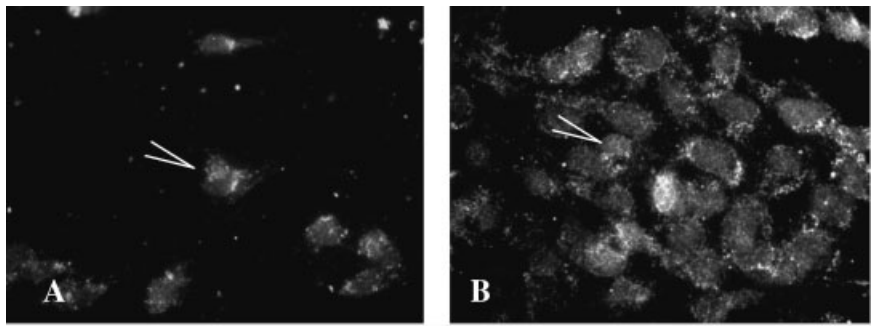


**Fig. 6.** Immunofluorescence of early and late passage mouse lens epithelial cells. **A** = early and **B** = late passeged αTN-4 cells.

young (3–11 years) and old (81–96 years) human donors exhibit changes in the nuclear architecture with prolonged passages and these alterations are comparable to nuclei obtained from progeria patients. It was also reported [Scaffidi and Misteli, 2006] that the cells obtained from young humans have significant levels of Lamin A/C present in the nucleoplasm, however, when one examines cells from old donors, it was found that the vest majority of Lamin A were accumulated in the nuclear rim. Thus it can be proposed that aging effect Lamin organization.

When we compared Lamin C of early and late passaged mouse lens epithelial cells, early passaged cells showed significantly prominent Lamin C band. The physiological significance of this requires further investigation. Fluorescence studies of Lamin C showed its presence in inner part of the nucleus and gave a punctuate appearance. It has been reported [Johnson et al., 2004] that Lamin A/C locate in the discrete nuclear foci in primary human cells and overlap with pRB in some areas, punctuate appearance of fluorescent indicates specific or discrete location of Lamins C in the tissue cultured mouse lens cells. Discrete location also suggest possible specific functional role of Lamin C in the mouse lens cells.

Goldman et al. [2005] reported that normal lamin organization is required for RNA polymerase II transcriptional activities and transcription factors such as retinoblastoma protein (pRB) and TATA binding proteins. It has also been suggested that premature aging processes could be result of alteration in the structural and functional properties of nuclear lamins and their binding proteins. Wilson [2005] reported that vertebrate Lamins and lamin associated proteins are involved in gene regulation, TGF-β signaling, DNA replication, mRNA transcription and also have fundamental roles in chromatin organization, nuclear architecture and nuclear assembly.

Data from studies with [Wilson et al., 2001, 2005] *C. elegance*, fruitful, xenopus eggs, and cultured mammalian cells suggest that gene expression, cell signaling, chromatin organization are directly or indirectly depend on the nuclear lamina organization.

It has been proposed that [Wilson, 2005] nuclear lamina filaments provide Scaffolds for the assembly, and sequestration of many proteins in the nucleus that regulate gene expression. We believe that the two most abundant nuclear matrix protein of lens epithelial cells, Lamin C and HSF-1 are probably associated with each other for their activities.

Verala et al. [2005] also reported that aging effects functional relationship between Lamin A/C and $P^{53}$. Johnson et al. [2004] showed that Lamin A/C controls the stability, localization and activities of pRB. Furthermore, both sterol regulatory element binding protein (SRE BP), and transcriptional repressor germ cell less are reported to interact with Lamin A/C.

Lamin A/C could suppress AP-1 function through diverse interaction with C-FOS [Ivorra et al., 2006] and could inhibit fibroblast proliferation by TGF-β1.

Thus it could be postulated that Lamin A/C not only participates in the formation of nucleoplasm organization, but this organization could modulate transcription of many essential regulatory molecules, and as aging effects structural organization of nucleus, many essential protein expressions are altered [Haithcock

et al., 2005]. We are examining possible interaction of Lamin C and HSF-1 proteins in the lens epithelial cells.

Our examination of NM proteins of mouse lens epithelial cells revealed two prominent proteins, Lamin C and heat-shock transcription factor-1. The possible interrelationship of these two proteins in aging and in maintenance of lens clarity needs further examination. Since HSF-1 gene expression is essential for lens homeostasis it is possible HSF-1 gene expression could be modulated by alterations in the nucleus matrix organization initiated by changes in the Lamin C structure.

## ACKNOWLEDGMENTS

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