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Nonchromatin Nuclear Proteins of Mammalian Lens Epithelial Cells

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Abstract The nuclear matrix (NM) proteins of six tissue cultured lens epithelial cell lines and one embryonic rabbit epidermal cell line were analyzed to determine possible tissue and species specificity of these proteins. The NM proteins were isolated by the modified Penman technique. The tissue cultured cells were pulsed with [^{35}S] methionine and nuclear matrix proteins were fractionated by two-dimensional (2-D) gel electrophoresis. The 2-D gels were dried and autoradiographed. The relative abundance of spot patterns of nuclear matrix proteins of different cells were compared. The data from these experiments revealed that all the examined cell lines have distinct spot patterns, however, all of NM profile showed a spot pattern in the 45 kDa region with acidic pH. Some of these spots cross-reacted with anti-vimentin antibodies, whereas a prominent protein spot in this region did not cross react with either vimentin or actin antibodies. The observed variations in the NM protein patterns of lens epithelial cells may reflect tissue and species specificity and also a role in the regulatory properties of these nuclear proteins in the eye tissue development. J. Cell. Biochem. 64:644–650.

Key words: nuclear matrix proteins; transgenic murine lens epithelial cells; vimentin; human transgenic lens epithelial cells

The mammalian lens epithelial cells are a group of specialized epithelial cells with numerous tissue specific functions. The majority of these cells maintain mitotic quiescence, however, retaining their ability to proliferate, if stimulated [Harding et al., 1971]. The lens epithelial cells also express tissue specific proteins, the crystallins [Piatigorsky and Zelenka, 1992]. The molecular basis of the control of division and the expression of tissue specific genes of these cells is not yet clearly elucidated. Recent reports in the literature suggested that the nuclear matrix (NM) proteins may play an important role in the control of gene expression [Stein et al., 1996; Mancini et al., 1996; Baskin, 1995]. It has been postulated that the three-dimensional organization of the nuclear matrix proteins maintain folding patterns of DNA, nucleosome organization, and subnuclear localization of both genes and transcription factors [Stein et al., 1996b]. Thus, allowing transcription factors accessibility or inaccessibility to the given genes at the optimum times [Stein et al., 1996a].

Stein et al. [1996b] clearly demonstrated, using osteoblasts and HeLa cells, that the NM proteins interact with regulatory sequences of DNA and transcription factors. Van Wijnen et al. [1993] proprosed that the NM proteins are ideal candidates for coordinating the program of sequential gene expression.

The control of division of lens epithelial cells and the eventual differentiation of epithelial cells to fiber cells is essential for the maintenance of lens transparency. Molecular events associated with these above mentioned activities probably are mediated via physical and chemical involvement of NM proteins. The tissue cultured mammalian lens epithelial cells provide an excellent model system to explore the above mentioned hypothesis and will also be a tool in understanding the regulatory mechanisms of gene expression in these cells.

In this study, six tissue cultured lens epithelial cell lines of human, mouse, and rabbit and one rabbit skin epidermal cell line were investigated. Three transgenic mouse lens epithelial

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cell lines (gifts from Dr. Paul Russel, NIH) [Yamada et al., 1990]; one human transgenic lens epithelial cell line (obtained from Dr. U. Andley, Washington University, St. Louis, MO) [Andley et al., 1994]; one normal mouse and one rabbit lens epithelial cell lines and a transformed rabbit skin epithelial cell line (ATCC, Rockville, MD) were used for these experiments. Both human and mouse transgenic lens epithelial cells were obtained by incorporation of α crystillin promoter in their genome. The NM proteins from each cell lines were isolated by modified Penman technique [Van Wijnen et al., 1993] and analyzed by Western blot and 2-D gel electrophoresis. The polypeptide profile of different cells lines were compared and all transgenic mouse lines showed increased protein synthesis activities. It is interesting to note that 2-D spot profiles of NM proteins of all cell lines contained polypeptide spots around 31 kDa to 45 kDa region. We believe that the NM proteins in this spot represent structural proteins present in all the cell lines examined and are essential in maintaining lens epithelial specific phenotype.

MATERIALS AND METHODS

Cell Culture

The lens epithelial cells were grown at 37°C in Dulbecco's medium supplemented with 20% fetal bovine serum (Gibco) in a humidified atmosphere of 5% CO_2 and 95% air. The confluent tissue cultured epithelial cells were exposed to [^{35}S] methionine (25 µci/ml) for 24 h.

Isolation of NM Proteins

The nuclear matrix proteins were extracted from confluent monolayer of lens epithelial cells growth in T-175 cell culture flasks. The procedure for nuclear matrix protein extraction was same as described earlier [Bagchi et al., 1995]. Briefly, after radioactive pulse ([^{35}S] methionine, 25 µci/ml) the cells were thoroughly rinsed with phosphate buffered saline (PBS) to remove all noncovalently bound radioactive methionine. The cells were then lysed in a cytoskeletal buffer (100 mM NaCl, 300 mM sucrose, 10 mM pipes buffer, 3 mM $MgCl_2$, 0.5% Triton X-100 and, 1.2 mM PMSF, pH 6.8). This procedure removes most of the soluble cytoplasmic proteins as supernatant after low speed centrifugation. The pellet was then treated with detergent buffer (0.5% deoxycholate, 1% Tween-40, 10 mM NaCl, 10 mM Tris-HCl, 3 mM $MgCl_2$, pH 7.4) for 20 min at 4°C and then centrifuged at 800 rpm for 10 min. This step removes most of the membrane and cytoskeletal proteins and associated polyribosomes. The pellet consists mostly of oval nuclei and associated intermediate filaments as confirmed by microscopy. The pellet was then digested with DNase-1(100 µg/ml) and RNase-A (25 µg/ml) in a digestion buffer (same as cytoskeletal buffer) for 30 min at room temperature (RT). Then 0.25 M ammonium sulfate solution was added to the digestion buffer. The last procedure releases chromatin bound proteins in the solution. After centrifugation for 10 min at 2,000 rpm at RT, the pellet was dissolved in 8M urea containing buffer and dialysed overnight in a buffer containing potassium chloride. The dialyzed sample was then centrifuged for 95 min at 100,000*g*. The supernatant contained mostly soluble nuclear matrix proteins, whereas pellet is predominantly composed of Lamins and other intermediate size filaments [Fey and Penman, 1988].

2-D Gel Electrophoresis

Two dimensional gel electrophoresis was performed according to the methods of O'Farrel [1975] using 10% SDS-PAGE and a pH range of 5.0 to 7.1 in the isoelectric focusing gel. The gels were stained with coomassie brilliant blue, photographed, and then enhanced with PPO/DMSO technique, dried, and exposed to kodak X-omat 5 films, for 7 days at −70°C. The X-ray films were developed, photographed, and analyzed.

Immunoblot of Nuclear Matrix Proteins

The nuclear matrix proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose membrane. Immunological assays were carried out using anti-vimentin antibodies. Briefly, following the transfer of NM proteins, membrane was incubated with the anti-vimentin antibodies at 4°C for several hours. After two to three washings, horseradish peroxidase-conjugated goat anti-rabbit immunoglobin G was added. Finally, antigen-antibody reaction was detected by using Bio-Rad-color reagent.

RESULTS

Figure 1 depicts SDS-Page profile of nuclear matrix proteins isolated from several lens epi-

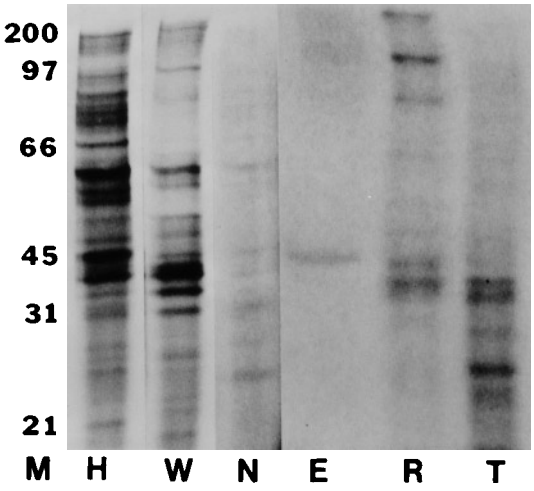

Fig. 1. SDS PAGE analysis of nuclear matrix proteins obtained from several tissue cultured lens epithelial cells and one skin epidermal cell line. All cells were pulsed with ^{35}S-methionine before protein extraction. After SDS-PAGE, gels were dried and autoradiographed using Kodak X-O mat film: The intensities of the bands indicate rate of polypeptide synthesis. M, Molecular weight marker; H, human transgenic lens epithelial cells; W, W518, transgenic mouse lens epithelial cells; N, NRK-12, transgenic mouse lens epithelial cells; E, embryonic rabbit epidermal cell; R, rabbit lens epithelial cell; T, TN4α, transgenic mouse lens epithelial cells.

thelial cells and skin cells of rabbit. The banding pattern of NM proteins of different cell lines show extensive variation, however, closer inspection reveals that there are polypeptide bands in the range of 45 kDa to 31 kDa region. Western blot analysis with anti-vimentin antibody suggest that some of these spots are either fragments of vimentin or vimentin binding proteins.

Figure 2a is the 2-D gel electrophoresis profile of NM proteins obtained from lens epithelial cells of transgenic mice (W518). This 2-D gel shows abundant spot patterns, however, major concentration of spots are localized in the 45 to 31 kDa region. Figure 2d is the gel profile of NM proteins of lens epithelial cells of another transgenic mice cell line NRK-12. The polypeptide spots of these NM protein are different from W518 (Fig. 2a), however, both groups of NM proteins have seveal spots around 45 and 31 kDa area. Figure 2b is the gel profile of NM protein obtained from transgenic mouse (TN4α). Like NRK-12 and W518, the polypeptide spot profile of TN4α shows major spots in the 45 to 31 kDa molecular weight region. Figure 2c is the gel profile of NM lens epithelial cells obtained from adult male balb/C mouse. The gel pattern depicts spots in 45 kDa region, however the total spot pattern is different from other three cell lines.

Figure 3 (a, b, c) show 2-D gel profiles of NM proteins obtained from rabbit lens, human transgenic lens, and rabbit skin epidermal cells. If one compares polypeptide spots of NM proteins obtained from two rabbit tissues with two lens epithelial cells, it is clear that all three NMs have polypeptide spots in the region of molecular weight 45 to 31 kDa. However, overall profile of NM proteins of rabbit and human lens cells and rabbit skin cells are significantly different.

Figure 4 shows the 2-D gel electrophoresis profile of NM protein obtained from lens cells cultured in presence or absence of 20% fetal calf serum. We used transgenic mouse lens epithelial cell lines (TN4α) and cell lines obtained from Nakano mouse lens for these experiments. Figure 4 (a, b) show gel profile of NM proteins obtained for TN4α cells cultured in serum containing and non-serum containing medium respectively. Both NM's protein profiles manifest spots at 45 to 31 kDa region.

Figure 4 (c, d) are NM protein profiles of NRK-12 cells incubated in presence (c) or absence (d) of fetal calf serum. Protein spot profiles of NRK-12 NM cultured in different condition show extensive variations, however, both groups of NM show distinct protein spots in the 45 to 31 kDa region. Figure 5 depicts the immunoblot of SDS-PAGE and 2-D gel electrophoresis profile of TN4α NM proteins. Both gel profiles present prominent antibody reaction at 36 kDa region. There is also reaction at about 70 kDa region.

DISCUSSION

The data obtained from these experiments show that the NM proteins extracted from similar tissues of different species and different tissues from same species show extensive variation indicating tissue and species specificity. However, 2-D gel profiles also manifest a commonality in all protein spot profiles. All seven NM proteins we examined, showed presence of polypeptides in the 45 to 31 kDa region. To identify and partially characterize some of the proteins in the 31 kDa and 45 kDa cluster, we employed western blot technique using antibodies against actin and vimentin. Actin was used as it is a known DNA binding protein, has a

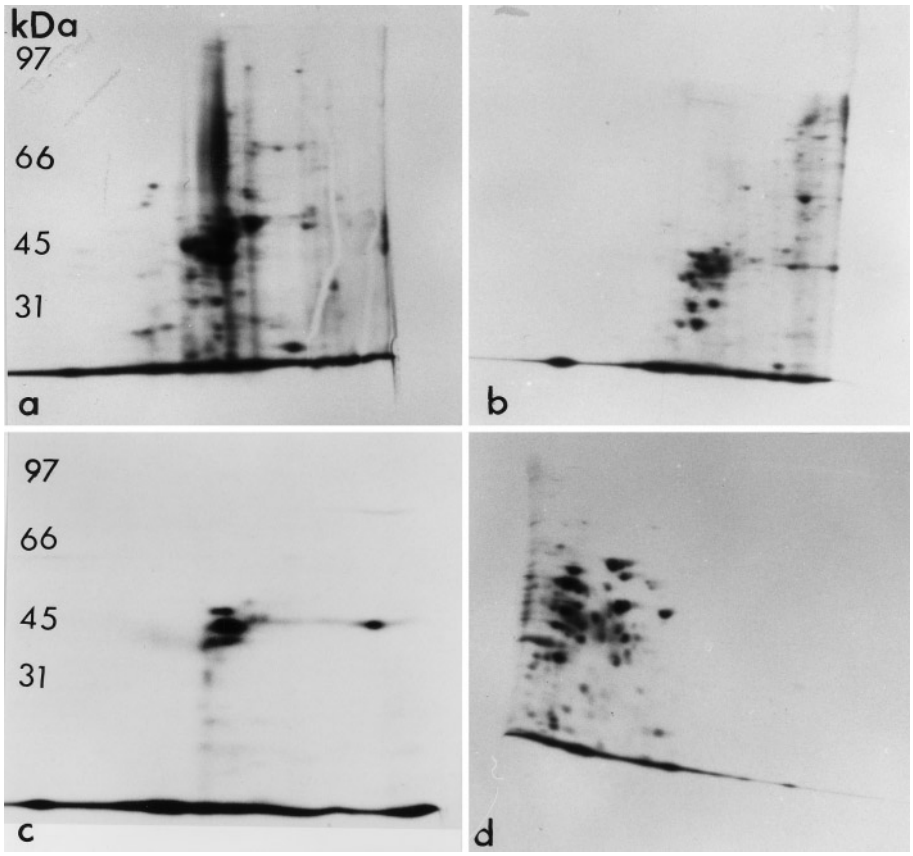

Fig. 2 Comparison of the protein composition of the nuclear matrix fractions derived from three transgenic mouse lens epithelial and one normal Balb/e mouse lens epithelial cells. The NM proteins were separated by 2-D gel electrophoresis and autoradiographed. **a:** W518; **b:** TN4α; **c:** Balb/c; **d:** NRK-12.

molecular weight of 43 kDa and shown to be present in NM of human tissues [Fey and Penman, 1988]. Vimentin, an abundent filamentous protein of lens epithelial cells was also reported to be able to interact with mouse genomic DNA [Wang et al., 1996]. Preliminary western blot analysis revealed no cross reactivity with actin antibodies, but vimentin antibodies manifested strong reactivity with NM proteins. However, the molecular weight of these cross-reacting proteins were significantly lower than the known molecular weight of intact vimentin.

Figure 2 showed the 2-D gel profiles of NM proteins obtained from three transgenic mouse lens epithelial cells and normal Balb/c mouse lens epithelial cells. Gel profile of these NM protein displayed similar spot pattern at 31 to 45 kDa region but also some significantly different profile. The spot profile of NMs obtained from transgenic mouse lens epithelial cells are extensive and different, an overall number of spots in the NM of these cells are significantly more than in NM of Balb/c mouse lens cells. It is probably indicative that the protein synthesis activity of the transgenic mouse lens cells are more extensive, which also correspond to the proliferative activity of these cells. The lens epithelial cells obtained from normal Balb/c have slower doubling rate even in presence of 20% fetal calf serum.

Thus, it seems that rapidly dividing cells also synthesize more and several nuclear matrix proteins. Increased level of nuclear matrix protein synthesis in the dividing cells also suggest a possible role of these proteins in the cellular proliferation [Feuerstein and Mond, 1987].

Figure 3 compares NM proteins of two rabbit tissues and NM proteins obtained from human and rabbit lens epithelial cells. This group of 2-D gels show that both the human and rabbit lens cells have prominent polypeptide spots in the 31 to 45 kDa region. The embryonic rabbit skin epithelial cell NM proteins showed a to-

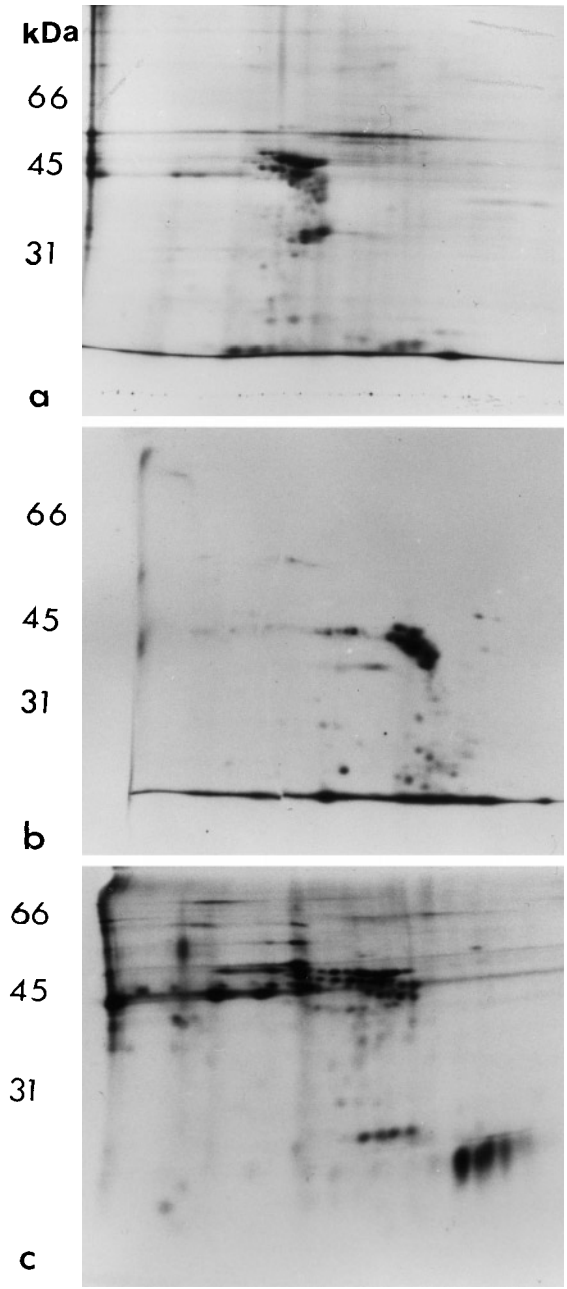

Fig. 3. Comparison of the nuclear matrix protein composition of tissue cultured epithelial cells derived from two tissue types. The cell lines examined were (**a**) rabbit lens epithelial cells, (**b**) human transgenic lens epithelial cells, and (**c**) embryonic rabbit epidermal cells. The nuclear matrix proteins were fractionated by 2-D gel electrophoresis technique.

tally different profile compared to lens epithelial NM. However, even NM profile of these cells have similar polypeptide spots in the 31 to 45 kDa range.

The 2-D gel profiles of NM proteins obtained from seven different cells lines manifested even with significant difference in the spot pattern, polypeptide spots in 31 to 45 kDa region. This indicates that these proteins are present in the nucleus of most of the cells. These experiments were performed with tissue cultured lens cells incubated in 20% fetal calf serum. The lens epithelial cells in vivo reside in serum free medium and do not actively proliferate. Thus we examined the effect of serum and proliferation on the protein spot pattern, especially of the 31 to 45 kDa protein(s).

Figure 4 clearly demonstrates that presence of serum in the culture medium resulted in significant increase in the synthesis of many NM proteins, however, even in the absence of serum in the culture medium, one can clearly observe protein spots in the 31 to 45 kDa range. It can be proposed that these proteins in the 31 and 45 kDa range may be important for the structural integrity of the nucleus and is present in nuclear matrix irrespective of its metabolic state. The immunoblot (Fig. 5) of 2-D gel profile of TN4α cells using anti-vimentin antibody clearly demonstrate that polypeptides in 37 to 40 kDa region are fragments of vimentin or vimentin-associated proteins. The presence of vimentin like proteins has been reported earlier by Fey and Penman [1988], using four human tissues. However, there are other polypeptide spots in the 45 to 31 kDa region, which do not cross react with vimentin. Some of these spots are under further analysis. Here we have reported presence of a group of prominent polypeptide spots present in all seven tissue cultured epithelial cells. Thus it can be postulated that these are possibly structural proteins, which are important for overall organization of chromatin.

The data presented in this manuscript also clearly demonstrate extensive variation of the NM protein profile obtained from different cell lines indicating tissue and species specificity. This implies possible functional role of some of these NM proteins. The increased synthesis of NM proteins in the presence serum containing medium also suggests role of these proteins in the gene regulatory events. In conclusion it is important initially to identify and catalogue both the prominent and minor proteins of nuclear matrix for better understanding of their possible role in the maintenance of 3-D architecture of the chromatin, and involvement in the control of tissue specific gene expression.

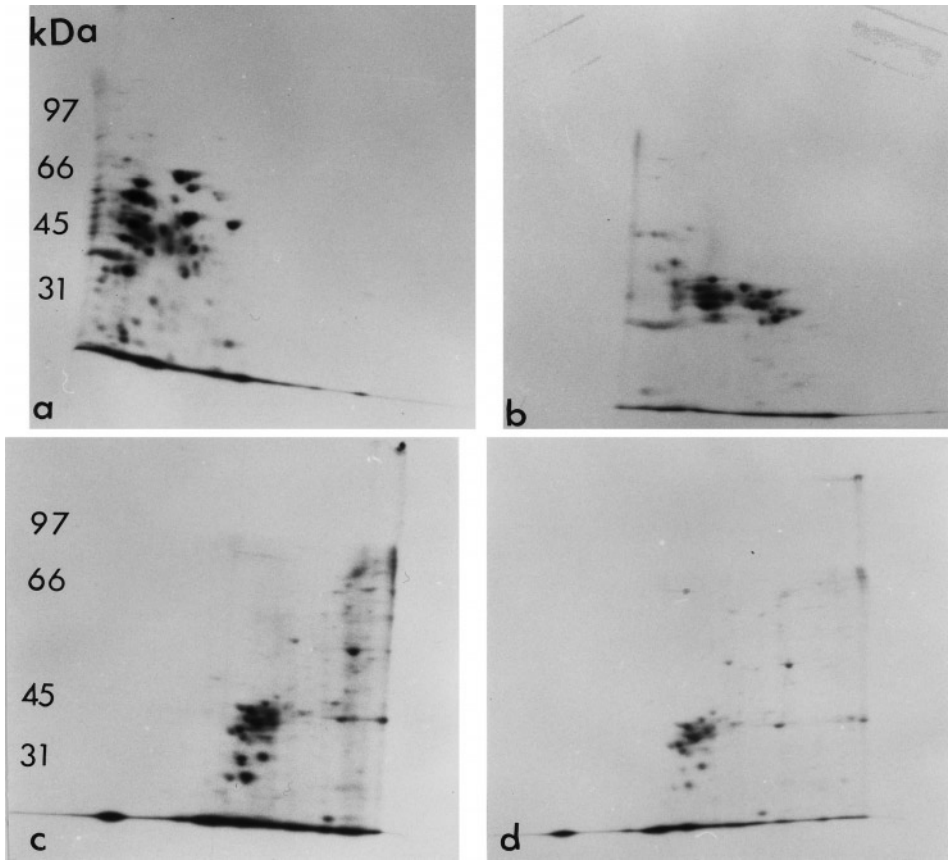

Fig. 4. Comparison of radioactive protein composition of two transgenic mouse lens epithelial cell lines incubated in either serum containing or serum free medium. The cell lines examined were NRK-12 (**a, b**), and TN4α (**c, d**). The nuclear matrix proteins were separated by the 2-D gel electrophoresis technique and autoradiograph. The protein profiles of cells incubated in serum containing medium are shown in panel c, whereas panel b and d represents cells cultured in serum free medium.

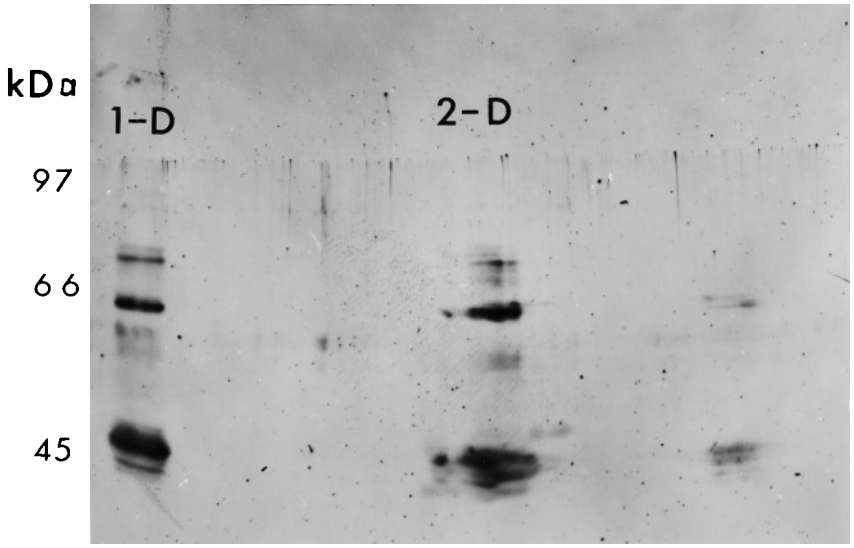

Fig. 5. Immounoblot of SDS-PAGE and 2-D gel electrophoresis patterns of nuclear matrix proteins obtained from transgenic mouse lens epithelial cells (TN4α). Polyclonal anti-vimentin antibodies were used for the immunoblot. 1-D, SDS-PAGE; 2-D, 2-dimensional gel electrophoresis.

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