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Similarity Between Nuclear Matrix Proteins of Various Cells Revealed by an Improved Isolation Method

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Abstract Comparative analysis of nuclear matrix proteins by two-dimensional electrophoresis may be greatly impaired by copurifying cytoskeletal proteins. The present data show that the bulk of adhering cytofilaments may mechanically be removed by shearing of nuclei pretreated with vanadyl ribonucleoside complexes. Potential mechanisms of action not based on ribonuclease inhibition are discussed. To individually preserve the integrity of nuclear structures, we developed protocols for the preparation of nuclear matrices from three categories of cells, namely leukocytes, cultured cells, and tissue cells. As exemplified with material from human lymphocytes, cultured amniotic cells, and liver tissue cells, the resulting patterns of nuclear matrix proteins appeared quite similar. Approximately 300 spots were shared among the cell types. Forty-nine of these were identified, 21 comprising heterogeneous nuclear ribonucleoproteins. Heterogeneous nuclear ribonucleoproteins L and nuclear lamin B2 isoforms were identified by amino acid sequencing and mass spectrometry. However, individually expressed proteins, such as the proliferating cell nuclear antigen, also pertained following application of the protocols. Thus, enhanced resolution and comparability of proteins improve systematic analyses of nuclear matrix proteins from various cellular sources. J. Cell. Biochem. 71:363–374, 1998.

Key words: nuclear matrix proteins; preparation method; two-dimensional polyacrylamide gel electrophoresis; heterogeneous nuclear ribonucleoproteins; vanadyl ribonucleoside complexes

The nuclear matrix is conceived as the intranuclear protein framework that determines the domain organization of the nucleus. Consisting of branched core filaments binding to DNA loops, it has been reported as playing a role in diverse structure-bound processes, such as DNA replication, DNA transcription, RNA processing, RNA transport, steroid hormone action, and signal transduction [for reviews, see Stuurman, et al., 1992; Berezney and Jeon, 1995].

In biochemical studies, the nuclear matrix has been defined as the insoluble material resisting sequential treatment of isolated nuclei with detergents, nucleases, and high ionic strength buffers [Berezney and Coffey, 1977]. Analysis of samples from different sources has revealed cell and tissue type-, differentiation-, cell state-, and tumor-specific nuclear matrix proteins [for reviews, see Stuurman et al., 1992; Berezney and Jeon, 1995]. The interest has also been focused on the group of nuclear matrix proteins among cells of various origins. Some of these common nuclear matrix proteins might represent the structural components of the nuclear matrix framework observed in the electron microscope [Fey et al., 1986; Penman, 1995].

For the detection of nuclear matrix protein components, two-dimensional polyacrylamide gel electrophoresis has served useful. However, restrictions are imposed on comparison of published patterns by varying experimental conditions used. In addition, major problems arise from persistent adherence of cytoskeletal proteins to isolated nuclei, leading to contamination of the final nuclear matrix probes [Kallajoki and Osborn, 1994].

In our effort to classify nuclear matrix proteins, samples from various human and rat tissues, cells, and cell lines [Holzmann et al., 1997; Korosec et al., 1997; Gerner et al., 1998; Gotzmann et al., 1997] are presently being analyzed. In practice, application of a standardized

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procedure for preparation of nuclear matrix protein samples from materials divergent as, for example, bladder cancer tissue and myeloid cells was not applicable. Especially isolation and purification of nuclei required individual protocols due to the varying stability of nuclear structures and the differing types and amounts of adhering cytofilaments.

In order to retain ribonucleoprotein complexes in nuclear matrix preparations, Fey et al. [1986] introduced vanadyl ribonucleoside complexes (VRC). The present results indicate that, beyond their role as RNase inhibitors, VRC affect physical properties of subcellular constituents in a manner which may be utilized to improve the purification of nuclear matrices. In experiments applying the improved method with different cell types, highly comparable patterns of nuclear matrix proteins resulted, cell type–specific nuclear matrix proteins still being recognizable. The described method, adapted alternatively for nucleated blood cells, cultured cells, and tissue cells, appears to be useful for systematic classification of nuclear matrix proteins.

MATERIALS AND METHODS

Cell and Tissue Samples

Leukocytes were prepared from palatine tonsils of children with acute tonsillitis by a standard protocol [Coligan et al., 1997]. The tissue was cut into small pieces, which were passed through a 250 µm steel sieve. The cell suspension was layered on Ficoll Paque and centrifuged. The lymph cells trapped in the interface were washed in Hanks' balanced salt solution (HBSS). The human amniotic cell line UAC [Lorenzetti et al., 1984] (cells were kindly provided by Dr. L. Snyers, University of Vienna) was cultured in Dulbecco's modified Eagle's medium (90%) fetal calf serum (10%). Human tissue specimens were frozen in dry ice and stored at -80°C.

Protocol I: Isolation of Nuclei From Leukocytes

The procedures were carried out at 4°C under the following general conditions. The buffers contained the protease inhibitors 1 mM PMSF, 1 µg/ml leupeptin, 1 µg/ml pepstatin A, and 1 µg/ml aprotinin (Sigma, St. Louis, MO). Both homogenization of cells and shearing of nuclei were performed with a tightly fitting glass-Teflon Potter homogenizer. Nuclei were resuspended with glass pipettes, carefully avoiding damages by shearing forces. Throughout all steps, nuclei were monitored by phase contrast microscopy.

Preparation of crude nuclei. Cells were washed twice in HBSS and lysed by suspension in buffer A (10 mM HEPES/NaOH, pH 7.4, 10 mM NaCl, 1 mM EGTA) supplemented with 3 mM $MgCl_2$ and 0.05% Nonidet P-40 (Sigma) [Antalis and Godbolt, 1991]. The nuclei were pelleted through a cushion of 300 mM sucrose in buffer B (50 mM HEPES/NaOH, pH 7.4, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EGTA) at 400*g* for 5 min.

Purification of nuclei. The nuclei were resuspended in 1.5 M sucrose in buffer B. The nuclei were sheared to remove adherent material. The suspension was layered on a 2 M sucrose cushion in buffer A and centrifuged at 6,000*g* for 20 min. The pellet was resuspended in 1 M sucrose in buffer B, adjusted to 2 mM VRC (Gibco BRL, Gaithersburg, MD) and incubated for 3 min. The nuclei were pelleted, resuspended in 0.2% sodium deoxycholate (Na-DOC) (Sigma), 0.4% Tween 40 (Sigma), 2 mM VRC, and 3 mM $MgCl_2$ in buffer A and incubated for further 3 min. The suspension was placed on a cushion of 0.3 M sucrose in buffer B, and the nuclei were collected by centrifugation at 700*g* for 5 min.

Protocol II: Isolation of Nuclei From Cultured Cells

Preparation of crude nuclei. General conditions were as in protocol I. Cells were washed twice in HBSS, suspended in 1 mM $MgCl_2$ in buffer A, and incubated at 4°C for 10 min. The swollen cells were homogenized. The suspension was adjusted to 0.35% Triton X-100, 3 mM $MgCl_2$, and 0.25 M sucrose and after 1 min incubation gently resuspended in a Potter. The crude nuclei were pelleted at 400*g* for 5 min through a 300 mM sucrose cushion in buffer B.

Purification of nuclei. The nuclei were suspended in 0.2% Na-DOC, 0.4% Tween 40, 2 mM VRC, and 3 mM $MgCl_2$ in buffer A and incubated for 5 min. Then the nuclei were centrifuged through a 300 mM sucrose cushion in buffer B at 700*g* for 5 min. The nuclei were resuspended in 1.6 M sucrose in buffer B, sheared in the Potter, and placed on top of a sucrose step gradient (2.0 M/2.3 M/2.5 M sucrose) in buffer B. After ultracentrifugation in a swingout rotor at 77,000*g* and 4°C for 60 min, the pellet was carefully suspended in 1 M sucrose in buffer B and the nuclei pelleted.

Protocol III: Isolation of Nuclei From Tissue Cells

Preparation of crude nuclei. General conditions were as in protocol I. Frozen tissue was

minced in HBSS into small pieces (approximately 1 mm^3) that were pressed through a 250 µm steel sieve. The suspension of lysed cells was filtered through a 70 µm nylon mesh and centrifuged through a 300 mM sucrose cushion in buffer B. The pellet was resuspended in 1.6 M sucrose in buffer B and homogenized. The suspension was placed on a 2.5 M sucrose cushion in buffer B and centrifuged at 6,000*g* for 30 min, yielding the crude nuclei in the interface.

Purification of nuclei. The crude nuclei were suspended in 2 mM VRC, 1 M sucrose in buffer B, incubated at 4°C for 3 min, and centrifuged. The nuclei were then resuspended in 0.2% Na-DOC and 0.4% Tween 40, 2 mM VRC, and 3 mM $MgCl_2$ in buffer A and incubated for 5 min. The nuclei were then collected by centrifugation through a 300 mM sucrose cushion in buffer B. The nuclei were resuspended in 1.6 M buffered sucrose and then sheared. The suspension was placed on top of a sucrose step gradient (2.0 M/2.3 M/2.5 M sucrose in buffer B) and ultracentrifuged in a swingout rotor as described in protocol II. The pellet was carefully suspended in 1 M sucrose in buffer B and the nuclei pelleted.

Preparation of Nuclear Matrices

General conditions were as in protocol I. By variation of the method of Fey and Penman [1988], the nuclei were suspended and incubated in slowly tumbling Falcon (Lincoln Park, NJ) tubes with 100 U/ml deoxyribonuclease I (DNase I, from bovine pancreas, EC 3.1.21.1; Sigma) in buffer C (50 mM PIPES pH 6.8, 300 mM sucrose, 50 mM NaCl, 3 mM $MgCl_2$, 0.25% Triton X-100, 2 mM VRC) at 22°C for 30 min. After centrifugation, the residual nuclear pellet was resuspended in 250 mM ammonium sulfate in buffer C and incubated at 4°C for 5 min. Finally, the pelleted nuclear matrix fraction was washed with 3 mM $MgCl_2$ in buffer A.

Two-Dimensional Gel Electrophoresis

High resolution two-dimensional gel electrophoresis was carried out by modification of the method of Hochstrasser et al. [1988] using the Protean II xi electrophoresis system (Bio-Rad, Richmond, CA). The nuclear matrix protein samples were dissolved in 10 M urea, 4% CHAPS, 0.5% SDS, and 100 mM DTT supplemented with 2% (v/v) ampholytes. Insoluble material was removed by centrifugation at $1.4 \times 10^5 g \times$ minutes. Isoelectric focusing of 70 µg protein samples was performed at 15,500 V-h in a stepwise fashion (2 h at 200 V; 3 h at 500 V; 17 h at 800 V) in 4% acrylamide (Gerbu, Gaiberg, Germany)/0.1% piperazine diacrylamide (Bio-Rad) in 1.5 mm × 16 cm tube gels. The gel buffer contained 0.035% Nonidet P-40 and 2% ampholytes (Merck, Darmstadt, Germany) (1 vol pH 3.5–10/1 vol pH 4–8/2 vol pH 5–7). Degassed 20 mM NaOH served as catholyte and 6 mM H_3PO_4 as anolyte. For SDS-polyacrylamide gel electrophoresis, the extruded tube gels were placed on top of 1.5 mm thick 10% polyacrylamide slab gels. After 3 min equilibration with 2.9% SDS, 70 mM Tris-HCl, and 0.003% bromphenol blue, the gels were run at 15°C in electrode buffer (0.1% SDS, 25 mM Tris-HCl, pH 6.8, 200 mM glycin). Gels were silver-stained by the method of Wray et al. [1981].

Identification of Protein Spots

Blotted spots of heterogeneous nuclear ribonucleoprotein (hnRNP) L and nuclear lamin B2 were identified by sequencing and matrix-assisted laser-desorption-ionization-time-of-flight (MALDI-TOF) mass spectrometry as reported previously for hnRNPs H and H′ [Holzmann et al., 1997]. In short, peptides formed by hydrolysis of blotted proteins with trypsin were separated by reversed-phase HPLC on a Vydac C_{18} column (1 mm × 250 mm) (Hewlett-Packard, Corvallis, OR) by means of the Hewlett-Packard 1090 HPLC Series II system. Selected fractions were analyzed by automated Edman degradation with the Hewlett-Packard G1005A protein-sequencing system. Peptide-mass fingerprinting of proteolytic digests was carried out by MALDI-TOF MS on a Hewlett-Packard G2025A MALDI-TOF/MS instrument. For Western blotting, proteins were electrophoretically transferred onto nitrocellulose membranes (0.2 µm). Actin and vimentin were identified immunologically as described before [Holzmann et al., 1997]. Cytokeratin subtypes were localized by comparison of spot patterns recognized by pan-keratin–specific antibodies with established 2-D patterns [Moll et al., 1988]. The monoclonal antibody GL-35 against nuclear lamins B1/B2 was a kind gift of Dr. K. Zatloukal (University of Graz, Austria). hnRNPs C, E, K, and M were recognized by their p*I*/molecular mass data and comparison to published nuclear matrix spot patterns [Mattern et al., 1996]. hnRNP F spots were recognized by the monoclonal antibody 7C2 specific,

as antibody 8A6, against hnRNPs F, H, and H′ [Matunis et al., 1994]. hnRNP L spots were recognized by the monoclonal antibody 4D11 [Piñol-Roma et al., 1989]. Both were kind gifts of Dr. G. Dreyfuss (University of Pennsylvania School of Medicine, Philadelphia, PA). hnRNP U was recognized by the polyclonal antibody α-SAF-A (K371) [Göhring et al., 1997], kindly supplied by Dr. F.O. Fackelmayer (Department of Biology, University of Konstanz, Germany). An affinity-purified antiserum pan-specific against human cytokeratins was a gift of Dr. P. Kovarik (University of Vienna, Austria). The monoclonal antibody against PCNA was purchased from BioMaker™ bm (Rehovot, Israel).

Evaluation of 2-D Data

Scanning of gels, spot editing, and evaluation of data was accomplished with the BioImage Investigator system (BioImage, Ann Arbor, MI), using the 2-D Analyzer™ (V 6.1) software package. Gels were edited to define spots, spot boundaries, and anchors. Identical spots were used as anchors to enable correct image overlay and coordinate transformation of spots by the software [Korosec et al., 1997]. For comparison by matching, six independent experiments with each cell type were performed. In the nuclear matrices of leukocytes, 358–412 spots were found; 378–409 spots were found in the nuclear matrices of amniotic cells and 367–422 spots in those of liver cells. Representative images are presented in Figures 3–5, respectively. For calibration, cellular proteins were used that are described in the human keratinocyte cell two-dimensional protein database [Celis et al., 1995]. The cells were kindly donated by Dr. J. Celis (Danish Centre for Human Genome Research, University of Aarhus, Denmark).

RESULTS

Effect of the Vanadyl Ribonucleoside Complexes on Nuclear Structures

VRC were used for inhibition of ribonuclease activity [Fey et al., 1986; Puskas et al., 1982], varying the experimental conditions for the preparation of nuclei and nuclear matrices. Apparently beyond or secondary to its function as an enzymatic inhibitor, VRC exerted effects which could advantageously be used for the isolation and purification of nuclei and nuclear matrices.

Accompanying control by phase contrast microscopy revealed that aggregates adhering to the surface of isolated nuclei were removed by the shearing of VRC-treated nuclei in a Potter homogenizer. The effect depended on the presence of VRC and was not evident after the sole exposure of nuclei to deoxycholate and Tween 40. In addition, two-dimensional electrophoresis revealed that, after shearing of nuclei in the presence of VRC, the bulk of cytokeratins was absent, otherwise imposing in the images of nuclear matrix proteins. Figure 1A,B depicts samples prepared from human bladder carcinoma cells particularly rich in cytokeratins.

Furthermore, the density was increased of nuclei that had been exposed to vanadyl ribonucleoside complexes. Consequently, nuclei could be pelleted through 2.5 M sucrose, allowing their separation from cytoskeletal proteins that were retained in the interphase. However, VRC-treated leukocyte nuclei were an exception, pelleting through 2.1 M sucrose but not through solutions of higher molarity.

In addition, VRC treatment prevented residual nuclear structures from aggregating during DNase treatment and salt extraction, procedures applied in the process of nuclear matrix preparation. This protective influence was most evident in experiments with leukocyte nuclei (Fig. 2B,C).

Apparently, leukocyte nuclei were affected by VRC in a specific manner. Lysis of leukocytes in hypotonic buffer in the presence of VRC induced a burst of nuclei. Leukocyte nuclei suspended in 1 M sucrose swelled significantly upon incubation with VRC while appearing undamaged in phase contrast microscopy (Fig. 2A). This may explain the above-mentioned behavior of leukocyte nuclei during ultracentrifugation through sucrose. Moreover, VRC-exposed leukocytes were more susceptible to damages induced by shearing and ultracentrifugation. Most likely these sensitivities are due to the minor concentration of nuclear lamins A/C in the nuclear lamina sublining and stabilizing the nuclear envelope of cells.

Protocols for the Purification of Nuclei From Three Categories of Cells

Nuclear matrices are routinely prepared by exposure of isolated nuclei to detergents, DNase I, and high ionic strength buffers. Depending on their origin, crude nuclei differ, in practice, by their stabilities and their contents of adhering cytoskeletal proteins. Because of this, individual schemes for their purification are

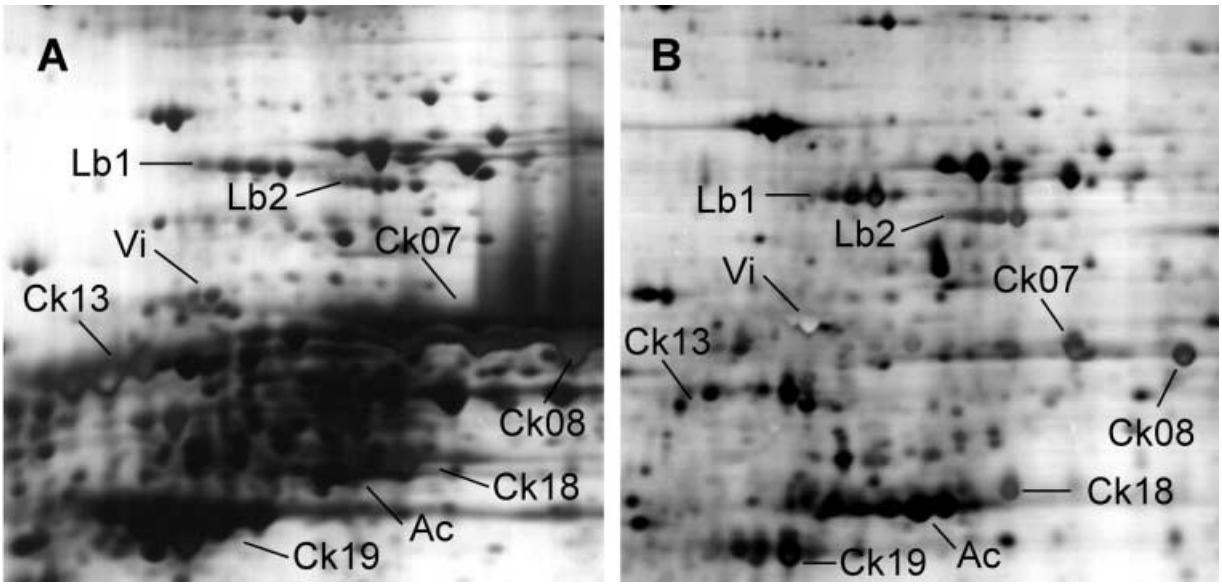

Fig. 1. Separation of cytoskeletal proteins by shearing of vanadyl ribonucleoside complex (VRC) treated nuclei. Two-dimensional pattern of nuclear matrix proteins of human bladder carcinoma cells. Scan of a silver-stained gel. Comparable regions (39–100 kDa, p*I* 4.0–5.5) of scanned silver stained gels. **A:** Control, VRC-exposed nuclei were not sheared but pelleted through 1.6 M sucrose. **B:** VRC-exposed nuclei were sheared and pelleted through 2.5 M sucrose. Other conditions as described in protocol III, Materials and Methods. Ac, actin; Ck, cytokeratins 7, 8, 13, 18, 19; Lb1, Lb2, nuclear lamins B1 and B2; Vi, vimentin.

recommended. Therefore, considering the above-mentioned experiences with vanadyl ribonucleoside complexes, methods were improved for the purification of nuclei and isolation of nuclear matrices. During the purification steps, the nuclear structures were continuously surveyed by phase contrast microscopy. Care was taken to minimize material adherent to nuclei, to avoid aggregation of nuclei and nuclear matrices, and to obtain samples yielding 2-D images of good quality. In the following, the rationale is outlined for the preparation of nuclei from fragile leukocytes and their subtypes, from cultured nonleukocyte cells and from tissue cells, respectively (for details, see Materials and Methods).

Protocol I was designed for the purification of nuclei from leukocytes (lymphocytes, monocytes, neutrophils) and derived cell lines. The nuclei are characterized by their low content of nuclear lamins A/C and low amounts of adherent cytoskeletal proteins. To avoid the above-described negative effects of VRC on leukocyte nuclear structure, we performed shearing of nuclei and sedimentation through a sucrose cushion in the absence of VRC at an early stage of purification. However, nuclei were later exposed to VRC in the presence of 1 M sucrose in order to preserve nuclear structures during the final stages of nuclear matrix preparation.

Protocol II was conceived for cultured cells expressing significant amounts of nuclear lamins A/C and of cytoskeletal proteins. The cultured cells originated from diverse normal and tumor cells and tissues. All cells examined contained actin, vimentin, and cytokeratins 8/18 and, depending on the cell type, other additional cytoskeletal proteins. Exposure to VRC in the presence of ionic and nonionic detergents allowed the subsequent dissociation of the bulk of attached cytofilaments from the nuclei by shearing. The density of nuclei having been increased by VRC, dissociated filaments were then removed by ultracentrifugation through 2.5 M sucrose.

Protocol III was applied for the purification of nuclei from tissue samples. In addition to the requirements outlined in the above protocols, it was necessary to remove any debris originating from the extracellular matrix. Therefore, crude nuclei, the density of which had not yet been increased by exposure to VRC, were collected from top of a 2.5 M sucrose cushion prior to other procedures.

Number of Nuclear Matrix Protein Spots Shared Among Different Cell Types

From a great number of experiments with cells of diverse origin, three are presented as

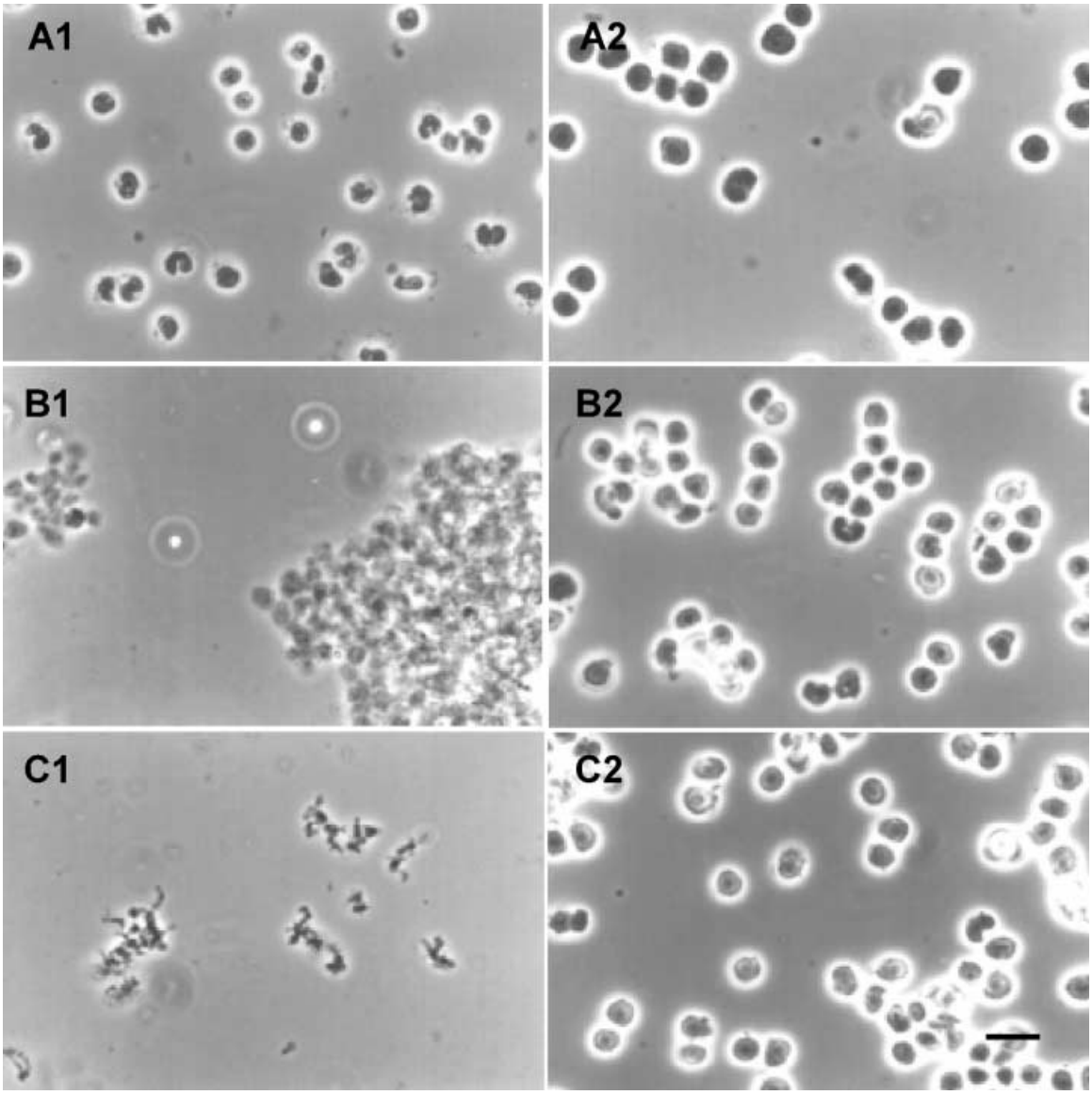

Fig. 2. Effects of VRC on leukocyte nuclear structures. Conditions as given in Materials and Methods for protocol I and nuclear matrix preparation. Incubations with 2 mM VRC. Phase contrast microscopy. Bar, 10 µm. A: Nuclei in 1 M sucrose. **A1:** Control. **A2:** Swelling in presence of VRC. B: DNase I–treated nuclei. **B1:** Aggregation in absence of VRC. **B2:** Aggregation prevented by pretreatment with VRC. C: DNase I– and ammonium sulfate–treated nuclei (i.e., final nuclear matrix). **C1:** Damaged nuclear structures in absence of VRC. **C2:** Damage prevented by inclusion of VRC.

being representative for application of the presently described protocols I–III. Figures 3–5 depict the protein spots of nuclear matrices isolated from human leukocytes, cultured amniotic cells, and liver tissue cells, respectively. The 2-D patterns were compared by computer-assisted image analysis. Spots identified by Edman degradation, mass spectrometry, or Western blotting are annotated.

Comparison of Figures 3–5 reveals a striking similarity between the overall spot patterns of nuclear matrix proteins. This is indicated by the number of spots shared among the samples isolated from significantly differing types of cells. When nine images were analyzed, three each of lymphocyte, UAC, and liver cell nuclear matrix proteins, 272 spots matched in nine of nine images and 298 in eight of nine images. Since the latter spots were also found in additional gels of the respective cell types, they were considered representative and marked by plus signs (Fig. 3–5). Many of the marked spots

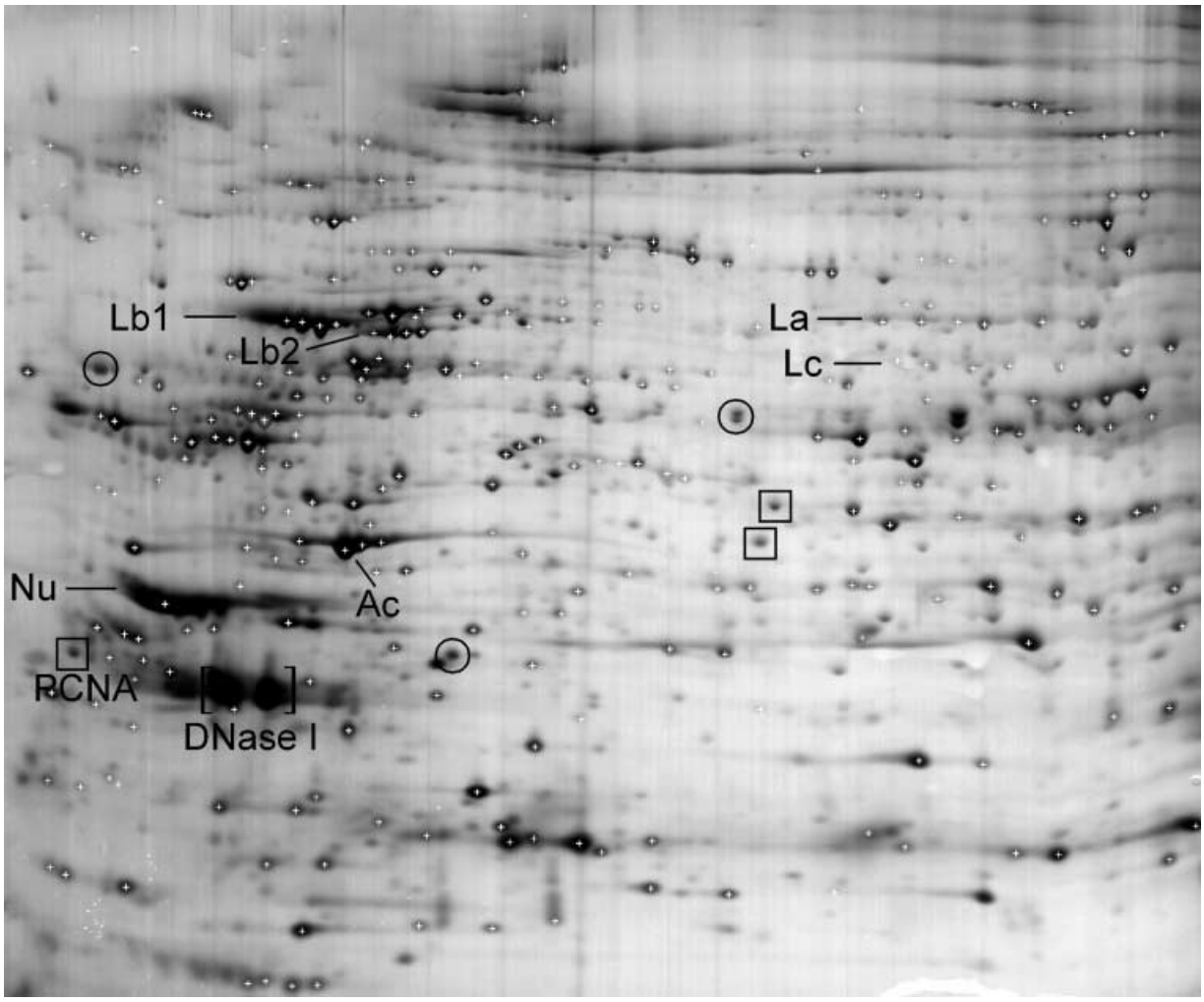

Fig. 3. Nuclear matrix proteins isolated from human leukocytes using protocol I. Protein identification as described in Materials and Methods. Ac, actin; La, Lb1, Lb2, Lc, nuclear lamins A, B1, B2, C; Nu, numatrin (B23); PCNA, proliferating cell nuclear antigen (in square). DNase I was added during preparation. Plus signs mark spots also present in the matrices of UAC and liver cells (Figs. 4, 5). Circles indicate spots present in Fig. 3 but not in Figs. 4, 5. Squares indicate spots present in Figs. 3 and 4 but not in Fig. 5.

represent ubiquitously occurring nuclear matrix proteins. However, their exact determination requires comparison of data from a greater number of different cell types, as will be presented elsewhere.

Identification of hnRNPs, Sequencing of hnRNP L

Heterogeneous nuclear ribonucleoproteins (hnRNPs) have earlier been recognized as main constituents of the nuclear matrix [Fey et al., 1986; Fey and Penman et al., 1988]. In a previous communication, we have reported about the identification of hnRNPs H and H′ as common nuclear matrix proteins by sequencing and mass spectrometry of the blotted proteins [Holzmann et al., 1997]. In the present study, a characteristic string of spots was characterized as isoforms of hnRNP L. Direct N-terminal sequencing of lymphocyte nuclear matrix proteins hL_1, hL_2, and hL_3 (Fig. 6) failed, suggesting that their N-terminal amino acids were blocked. Analysis of four tryptic peptides of protein L_1 yielded the sequences TDNAGDQxG, ISRPGDSDDS, MxPPVGGHR, and IEYA, corresponding to aa at positions 31–39, 148–157, 314–322, and 233–236 of hnRNP L [Piñol-Roma et al., 1989] (SwissProt account P14866). Analysis of tryptic digests of the blotted proteins by MALDI-TOF/MS revealed 20 mass fragments common to proteins L_1, L_2, and L_3. Eleven of these matched calculated hydrolysis products (not shown). Furthermore, proteins L_1 (65

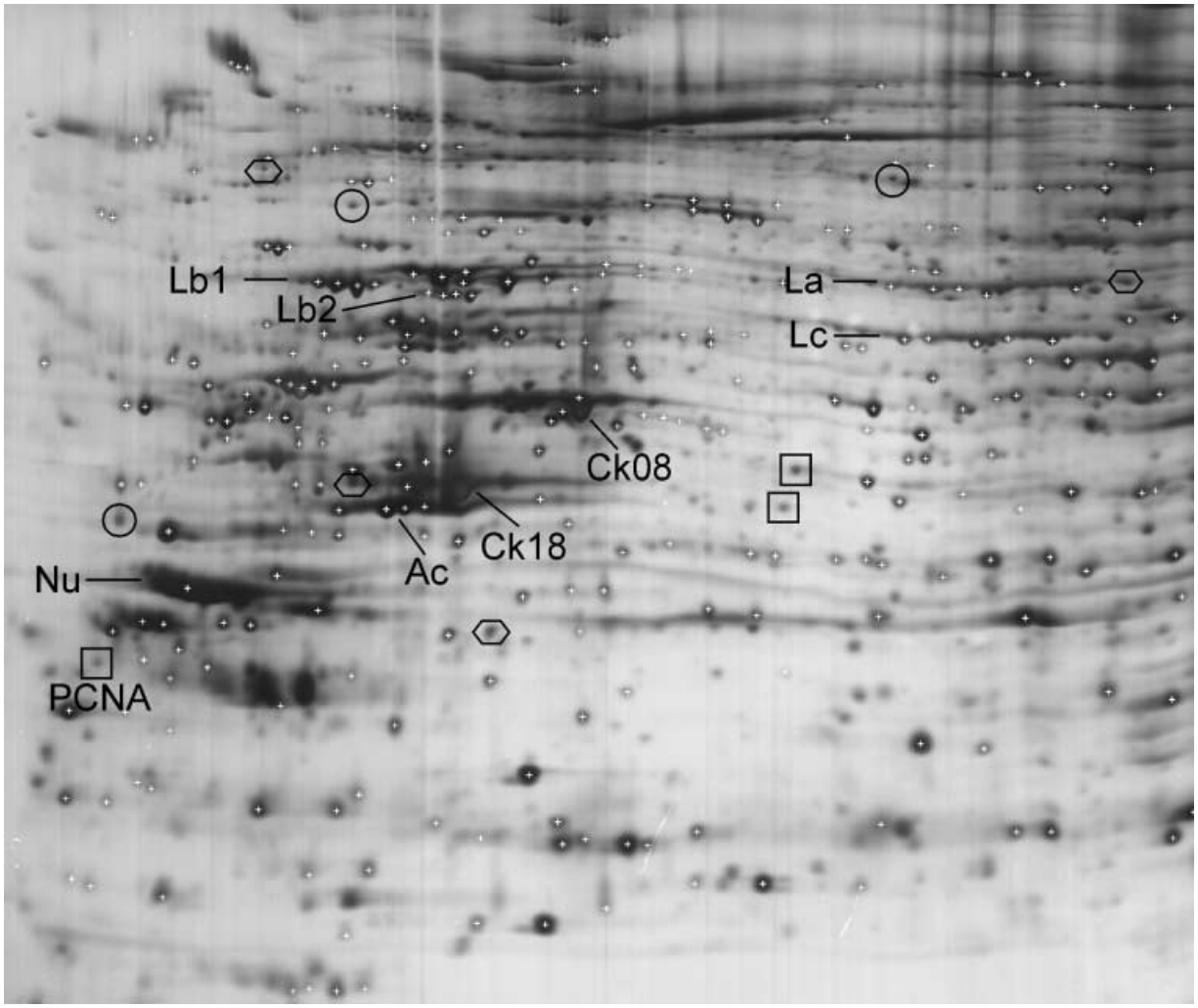

Fig. 4. Nuclear matrix proteins isolated from cultured UAC cells using protocol II. Annotation as in Figs. 1 and 3. Circles indicate spots present in Fig. 4 but not in Figs. 3 and 5. Squares indicate spots present in Figs. 3 and 4 but not in Fig. 5. Hexagons indicate spots present in Figs. 4 and 5 but not in Fig. 3.

kDa, p*I* 7.6), L_2 (65 kDa, p*I* 7.4), L_3 (65 kDa, p*I* 7.2) and L_4 (65 kDa, p*I* 6.9) were recognized by a monoclonal antibody specific for hnRNP L [Piñol-Roma et al., 1989], indicating that the four spots represent isoforms of the protein. Other hnRNP components were identified as specified in Materials and Methods. The final pattern of 22 hnRNP spots consisted of members of the hnRNP C, E, F, H, K, L, M, and U families. The spots were found in each of the analyzed nuclear matrix samples, the pattern being analogous to that recently described by Mattern et al. [1996] (Fig. 6).

Identification of Filamentous Proteins, Sequencing of Nuclear Lamin B2 Proteins

The antibody available for the recognition of nuclear lamin B2 also interacts with nuclear lamin B1. In order to unequivocally establish the position of nuclear lamin B2 in 2-D images of nuclear matrix proteins, we sequenced blotted proteins. Analysis of tryptic digests revealed the internal sequences EGELTVAQ, LELEQTYQ, and TVLVNAD for spot $Lb2_1$ (64 kDa, p*I* 5.45) (Fig. 6). The sequences were identical to those of human lamin B2 at positions 35–42, 172–179, and 457–463 [Biamonti et al., 1992] (Swissprot account Q03252). The peptides YGGPATPLS and LAHYIDR could not be correlated, since the N-terminal human sequence has not yet been communicated [Biamonti et al., 1992]. However, these sequences corresponded 78% and 100%, respectively, to the aa sequences 7–15 and 35–41 within the N-terminal region of the published complete mouse nuclear lamin B2 sequence [Hoger et al.,

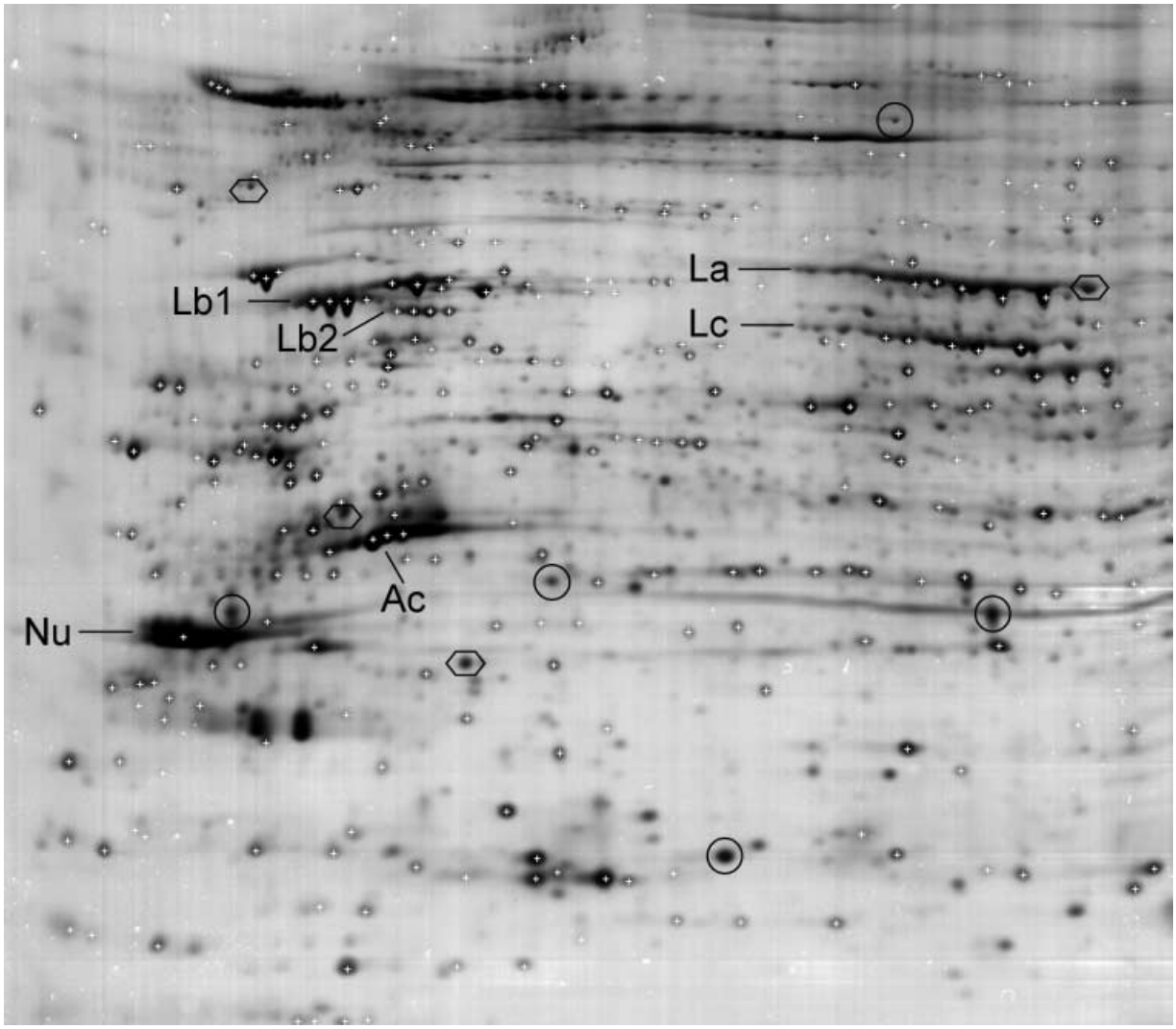

Fig. 5. Nuclear matrix proteins isolated form human liver tissue using protocol III. Annotation as in Fig. 3. Circles indicate spots present in Fig. 5 but not in Figs. 3 and 4. Hexagons indicate spots present in Figs. 4 and 5 but not in Fig. 3.

1990] (Swissprot account P21619). Thus, the present analysis also provides new information on the N-terminus of human lamin B2. Mass spectrometry of tryptic digests of spots $Lb2_1$ and $Lb2_2$ (64 kDa, p*I* 5.4) revealed 15 common mass fragments that corresponded to calculated tryptic fragments of human nuclear lamin B2 (not shown). Finally, spots $Lb2_1$, $Lb2_2$, $Lb2_3$ (64 kDa, p*I* 5.35), and $Lb2_4$ (64 kDa, p*I* 5.3) were recognized by the monoclonal antibody specific for nuclear lamins B1 and B2. The results thus demonstrate that the string of proteins marked as Lb2(1–4) in Figure 6 represent isoforms of the filamentous protein. The nuclear lamins A/C and B, actin, and vimentin spots were detected as described in Materials and Methods.

In summary of the last two paragraphs, from a total of 298 individual nuclear matrix protein spots shared among the three different cell types, 49 were identified. Their position is shown in the computer-generated composite image, derived by matching of nine gels, using three of each cell type (Fig. 6).

Cell Type–Specific Nuclear Matrix Proteins Are Preserved After Application of the Three Protocols

Individual characteristics of the spot patterns of different cell types were maintained,

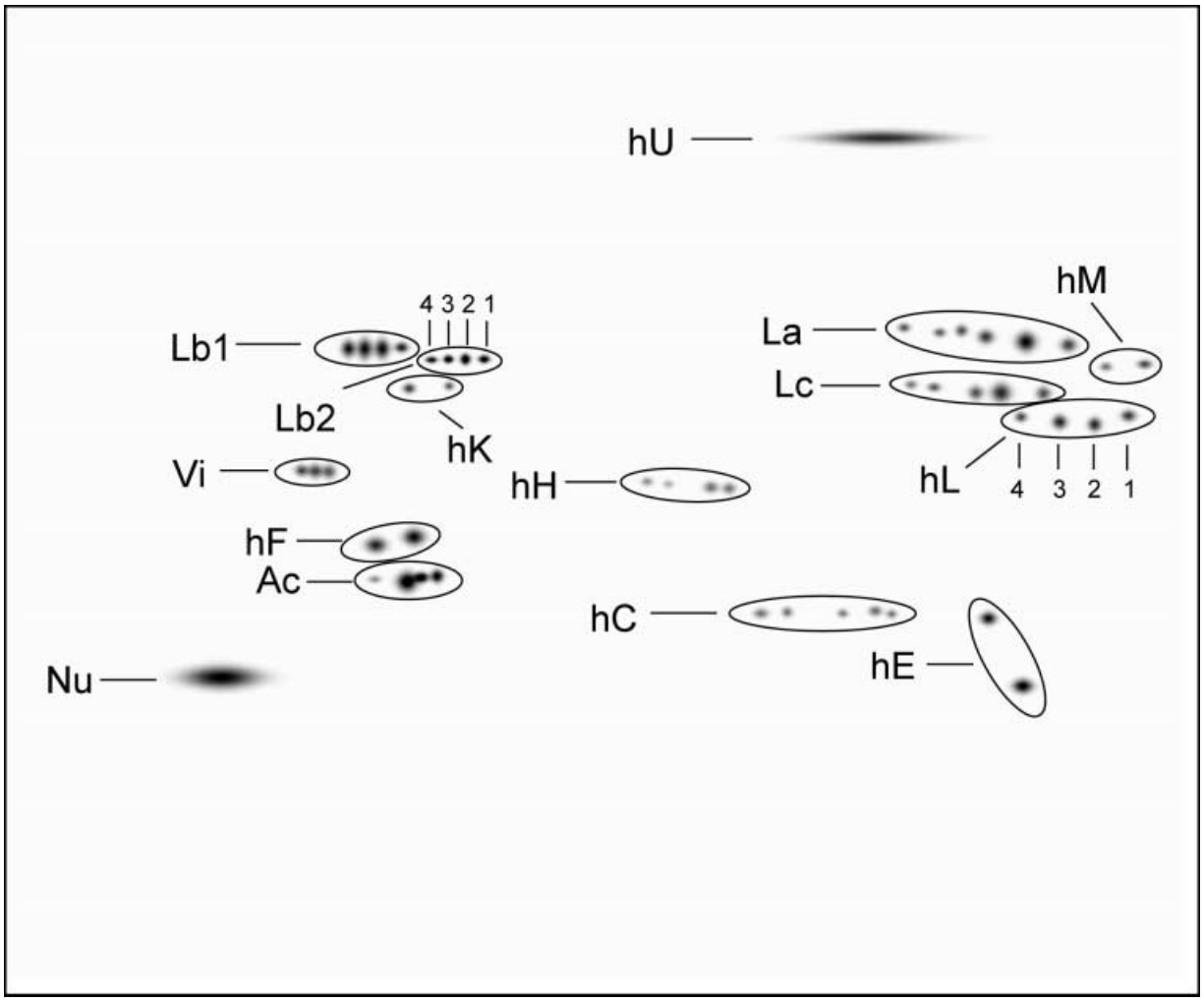

Fig. 6. Computer-generated 2-D image of identified nuclear matrix protein spots present in each of the three cell types. Ac, actin; hC, hE, hF, hH, hK, hL, hM, hU, heterogeneous nuclear ribonucleoproteins C, E, F, H, K, L, M, U; La, Lb1, Lb2, Lc, nuclear lamins A, B1, B2, C; Nu, numatrin (B23); Vi, vimentin.

following the isolation of nuclear matrix proteins by the three protocols. Thus, the proliferating cell nuclear antigen (PCNA) was present in the matrices of the dividing cultured UAC cells (Fig. 4) and leukocytes of inflamed tonsils (Fig. 3). It was, however, not apparent in the nuclear matrix of liver cells mainly consisting of nondividing cells (Fig. 5). Moreover, some spots (encircled in Figures 3–5) were present solely in the nuclear matrix of one cell type. Other spots were found only in two of the three images. Thus, the spots shown in squares were present only in lymphocyte and UAC cell samples (Figs. 3, 4), while the spots marked by hexagons were present in liver and UAC cells (Figs. 4, 5). The finding of such characteristic nuclear matrix proteins will allow further classification of proteins specific for individual cells and tissues, cell states, or malignancy.

DISCUSSION

The presence of vanadyl ribonucleoside complexes in the reaction mixtures exerted dual effects on isolated nuclear structures. First, it preserved the integrity of nuclear matrices, especially of matrices lacking nuclear lamins A/C, as those of leukocytes (Fig. 2). This is in line with the considerations of Fey et al. [1986], who introduced VRC as RNase inhibitors in order to obtain RNP-containing nuclear matrices. It has also been suggested that the isolated nuclear matrix may require intact RNA for structural integrity [Nickerson et al., 1989; Fey et al., 1986].

Second, it was observed that VRC treatment allowed the separation of adhering bulk cytokeratins by shearing of nuclei, an effect unlikely to be caused by inhibition of ribonuclease activity. Apparently, VRC affect the stiffness or other physical properties of adherent filamentous structures, rendering them more vulnerable to mechanical disruption. A study of literature reveals that the nucleoside-complexed and -uncomplexed vanadyl (oxovanadium (IV)[VO^{++}]) and vanadate (oxovanadium (V)[VO_3^-]) ions (V_i), capable of equilibrating dependent of the redox conditions [Lienhard et al., 1972], inhibit enzymes catalyzing phosphoryl or nucleotidyl transfers [Puskas et al., 1982; Lienhard et al., 1972; Goodno, 1979]. Thus, vanadyl ribonucleoside complexes have been described as analogs of nucleotide transition states that are occurring during the phosphodiesterase reaction catalyzed by many ribonucleases [Puskas et al., 1982; Lienhard et al., 1972]. On the other hand, VRC and V_i have been found to interact with active sites of kinases and phosphatases not involved in nucleic acid metabolism [Goodno, 1979]. In context with the present topic, it is of interest whether nucleotide binding cytoskeletal proteins also interact with vanadium compounds. Indeed, a vanadate-dependent inhibition of myosin ATPase [Goodno, 1979, 1982], dissociation of the actomyosin complex [Goodno and Taylor, 1982], and trapping of an ADP analog into the ATPase site of heavy meromyosin or myosin subfragment 1 [Sutoh et al., 1986], have been reported. It is also of interest that ATP/ADP-dependent alterations of the physical properties of actin polymers have been observed [Janmey et al., 1990], the monomers consisting of adenine nucleotide binding proteins with ATPase activity. It remains further to be investigated whether direct interaction of VRC with structural proteins is the base for the observed dislocation of filaments from nuclei.

In our experience, use of cell-type adapted preparation methods and continuous control of nuclear structures by phase contrast microscopy essentially influenced the quality of the results. Removal of bulk cytofilaments improved the reproducibility and comparability of the 2-D protein patterns. In effect, it also markedly increased the resolution of the proteins in the gels, thus facilitating the classification of nuclear matrix proteins.

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