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Major Internal Nuclear Matrix Proteins Are Common to Different Human Cell Types

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Abstract

The nuclear matrix may be involved in the structural and functional organization of the cell nucleus. However, we still do not understand the molecular basis of the intranuclear fibrogranular network that is part of the nuclear matrix. We recently described a method to identify internal nuclear matrix proteins [Mattern et al. (1996): J Cell Biochem 62:275–289], which was done by comparing two nuclear matrix preparations: one with and one without the internal structure by using quantitative two-dimensional gel electrophoresis. In the present study, we use the same approach to compare the nuclear matrix proteins of four different human cell types to investigate whether they have a similar internal nuclear matrix protein composition. Major nuclear matrix proteins present in all these cell types likely represent the base of the internal nuclear matrix. We demonstrate that the 25 most abundant internal nuclear matrix proteins are common to all four cell types. Together, these common proteins represent more than 75% of the total internal nuclear matrix protein mass in each cell type. This set of proteins includes B23 and most hnRNP proteins. The quantity of most of these proteins is very similar in the four cell types. The fact that the internal nuclear matrix consists mainly of hnRNP proteins, which may be involved in transcription, transport, and processing of hnRNA, supports the idea that the internal nuclear matrix is the result of these processes. J. Cell. Biochem. 65:42–52. © 1997 Wiley-Liss, Inc.

Keywords: nuclear matrix; human cell types; 2-D gel electrophoresis; heterogeneous nuclear ribonucleoproteins; B23

The nuclear matrix is the structure that remains when most of the chromatin and loosely bound components are removed from the interphase nucleus [Berezney and Coffey, 1974]. This structure consists of the nuclear lamina, containing the nucleopores, and an intranuclear fibrogranular network, containing residual nucleoli. The molecular basis of the internal matrix is poorly understood [Cook, 1988; Mattern et al., 1997; Stuurman et al., 1992]. Many components of the molecular machinery for transcription, RNA processing, and replication are part of the nuclear matrix [for reviews, see Berezney et al., 1995; Getzenberg, 1994; Jackson and Cook, 1995; Nickerson et al., 1995; Van Driel et al., 1995; Verheijen et al., 1988]. Most of the nuclear RNA remains associated with the nuclear matrix, if no RNase is used during isolation [He et al., 1990; Huang et al., 1994; Van Eekelen and Van Verrooj, 1981; Wansink et al., 1996]. Specific genomic sequences, called scaffold/matrix-associated regions (S/MARs), bind specifically to the nuclear matrix and may function as boundary domains of independently regulated chromatin loops [Bode et al., 1995; Cockerill and Garrard, 1986; Klehr et al., 1991; Ludérus et al., 1992; Mirkovitch et al., 1984]. Active genes are also bound to the nuclear matrix [Ciejek et al., 1983; Gerdes et al., 1994; Jackson and Cook, 1985]. Therefore, the nuclear matrix may play an important role in the structural and functional organization of the cell nucleus.

The protein composition of nuclear matrix preparations is complex partly due to cytoskeletal structures, predominantly intermediate filaments that are associated with it. By comparison of intact nuclear matrix preparations containing the internal fibrogranular structure with nuclear shell preparations, a nuclear matrix fraction lacking this internal structure [Ludérus et al., 1992], we have been able to distinguish the internal nuclear matrix proteins from cytoskeletal proteins and from proteins of the nuclear lamina-pore complex [Mattern et al., 1996]. In this way, we have identified the 21 most abundant internal nuclear matrix proteins...
proteins of HeLa S3 cells, together representing about 75% of the total protein mass of the internal fibrogranular nuclear matrix structure. This set of proteins includes the predominantly nucleolar protein B23 (numatrin) and 16 hnRNP (heterogeneous nuclear ribonucleoprotein) proteins.

It is likely that components that constitute the basal structure of the internal nuclear matrix are abundant and occur in many different cell types. Stuurman et al. [1990] showed that cells of different origin have many nuclear matrix proteins in common. In addition to common proteins, many investigators have reported the presence of cell-type-specific proteins in nuclear matrix preparations. Changes in the protein composition of nuclear matrix preparations have been observed with differentiation [Dworetzky et al., 1990; Stuurman et al., 1989] and transformation [Bidwell et al., 1994; Getzenberg et al., 1996; Keesee et al., 1994; Khanuja et al., 1993; Partin et al., 1993]. However, it is not clear whether these changes reflect changes in the internal nuclear matrix. Often, these differences in nuclear matrix composition are due to quantitatively minor proteins.

In this study, we compare the nuclear matrix protein composition of four different human cell types (cervix carcinoma, bladder carcinoma, myelogenous leukemia, and embryo carcinoma cells) using quantitative two-dimensional (2-D) gel electrophoresis. We have identified, in each cell line, the most abundant internal nuclear matrix proteins, i.e., proteins that individually contribute more than 1% to the total protein mass of the internal nuclear matrix. Together, 25 proteins meet this criterion. Remarkably, all these proteins were present in each of these four cell types. Also, the relative amount of most proteins was quite similar. This set included B23 and almost all known hnRNP proteins. Most of these 25 proteins interact with RNA directly or indirectly. B23 is probably involved in the processing of rRNA and may also be involved in the assembly and transport of ribosomes [Borer et al., 1989; Herrera et al., 1995]. The hnRNP proteins may play an important role in the packaging, processing, and transport of hnRNA (including pre-mRNA) [Dreyfuss et al., 1993; Kiledjian et al., 1994]. These RNA–protein complexes, which form the internal nuclear matrix, evidently are important for the structural and functional organization of the nucleus.

MATERIALS AND METHODS

Cell Culture

HeLa S3 (human cervix carcinoma) cells were grown as suspension culture in roller bottles in 10% CO2-saturated, MiliQ's modified minimum essential medium (Gibco, Paisly, UK) containing 5% (v/v) heat-inactivated fetal calf serum (Gibco). T24 (human bladder carcinoma) and NT2/D1 (human embryo carcinoma) cells were grown in monolayer under a 10% CO2 atmosphere in Dulbecco's modified Eagle medium (Gibco) containing 10% (v/v) heat-inactivated fetal calf serum. K562 (human myelogenous leukemia) cells were grown in suspension under a 5% CO2 atmosphere in RPMI 1640 (Gibco) containing 10% (v/v) heat-inactivated fetal calf serum. All cells were grown at 37°C, and media were supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin (Gibco).

Isolation of Nuclear Matrices and Nuclear Shells

Nuclear matrices and nuclear shells, i.e., nuclear matrices without the internal fibrogranular structure, were isolated as described by Mattern et al. [1996]. All incubations were carried out at 0–4°C at a cell density of 5 × 10⁷ cells/ml, unless stated otherwise. For nuclear matrix isolation, cells were washed twice with phosphate buffered saline (PBS) and collected by centrifugation at 400 g for 5 min. The cells were then extracted for 5 min in CSK100 buffer [10 mM PIPES, pH 6.8, 0.3 M sucrose, 100 mM NaCl, 3 mM MgCl₂, 5 U/ml RNasin (Promega, Madison, WI), 1 mM EDTA, 1 mM PMSF, 1 µg/ml leupeptin] containing 1% (w/v) Triton X-100 plus extra 15 U/ml RNasin. The nuclei were subsequently sheered by forcing them five times through a 22-gauge needle. After centrifugation for 5 min at 400g, nuclei were incubated for 30 min in CSK100 buffer containing 0.5 mM sodium tetraphosphate for stabilization of the internal nuclear matrix. Nuclei were washed twice with CSK50 buffer (same composition as CSK100 buffer but with 50 mM NaCl instead of 100 mM) by centrifugation and were then digested at a density of 2 × 10⁸ nuclei/ml in the same buffer containing 500 U/ml RNase-free DNase I (Boehringer, Mannheim, Germany) plus 15 U/ml RNasin for 30 min at 25°C. Subsequently, ammonium sulfate in CSK50 was added dropwise to a final concentration of 0.25 M. After incubation for 15 min, nuclear matri-
ces were pelleted by centrifugation at 1,000g for 5 min and washed once with CSK50.

Nuclear shells were isolated in the same way as the nuclear matrices with the following modifications. RNasin and sodium tetrathionate were omitted, and 1 mM dithiothreitol (DTT) was added to all buffers. In addition, matrices were digested with 50 µg/ml RNase A at a density of 1 × 10^9 matrices/ml in CSK50 buffer for 15 min at 25°C. Subsequently, matrices were extracted for 15 min by adding NaCl to a final concentration of 2 M and DTT to a final concentration of 40 mM. Nuclear shells were collected by centrifugation at 14,000g for 20 min and washed once with CSK50.

Two-Dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis was performed as described by Celis et al. [1993], with some minor modifications. Samples were freeze-dried before solubilization in lysis buffer, and 1% (w/v) CHAPS was added to this lysis buffer and the first-dimension gel medium. Isoelectric focusing (IEF) gels contained 2% ampholytes (0.67%, pH 3–10; and 1.33%, pH 5–8; BioRad, Richmond, CA) and were run for 1 h at 200 V, 1.5 h at 400 V, and 16 h at 700 V. Nonequilibrium pH gradient electrophoresis (NEPHGE) gels contained 2% ampholytes (0.67%, pH 3–10; 0.67%, pH 5–8; and 0.67%, pH 7–9; BioRad) and were run for 1 h at 200 V and 4 h at 700 V. For the second dimension, 10% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) were used. Gels were silver stained as described by Rabilloud [1992], with some modifications [Mattern et al., 1996]. The gels were scanned using a Molecular Dynamics laser scanner (Kent, U.K.). PDQUEST software (PDI, New York, NY) was used for quantitative analysis of the gels. Standards for 2-D SDS-PAGE (BioRad) were used to estimate the apparent molecular weight (M_r) and isoelectric point (pI). Protein spot identification by immunoblotting, comigration, microsequencing, and comparison with the 2-D gel database of Celis et al. [1994] has been described by Mattern et al. [1996].

RESULTS

Protein Composition of Nuclear Matrices and Nuclear Shells Isolated From Different Human Cell Types

To investigate whether the protein composition of the internal nuclear matrix is similar across different cell types, we isolated nuclear matrices and nuclear shells from four human cell types: (1) HeLa S3 (cervix carcinoma) cells, (2) T24 (bladder carcinoma) cells, (3) K562 (myelogenous leukemia) cells, and (4) NT2/D1 (embryo carcinoma) cells. Nuclear matrices were isolated from sodium tetrathionate-stabilized nuclei by DNase I digestion followed by 0.25 M ammonium sulfate extraction. Nuclear shells were prepared like nuclear matrices but without the sodium tetrathionate stabilization, followed by dissociation of the internal nuclear matrix by RNase digestion and extraction with 40 mM dithiothreitol and 2 M NaCl [Belgrader et al., 1991; Fey et al., 1986; Kaufmann and Shaper, 1984; Mattern et al., 1996]. All isolations were carried out in duplicate. The protein composition of the preparations was analyzed by 2-D gel electrophoresis by using IEF or NEPHGE in the first dimension and SDS-PAGE in the second dimension (Figs. 1–4). Each sample was run in duplicate, so four gels of each preparation type were available for analysis.

Visual inspection showed that many nuclear matrix proteins are common to all four cell types. The most striking differences between the cell types concerned cytoskeletal proteins, present both in nuclear matrix and in nuclear shell preparations. Vimentin, an intermediate filament protein, is abundantly present in the cell types HeLa S3, K562, and NT2/D1 but absent from T24 cells. Keratin 17, another intermediate filament protein, is present in HeLa S3 and T24 but absent in K562 and NT2/D1. The amount of actin is considerably less in K562 than in all other cell types. Previous studies have shown that cytoskeletal structures co-isolate with nuclear matrix preparations. The intermediate filaments are especially tightly associated to the nuclear lamina [Capco et al., 1982; Kallajoki and Osborn, 1994; Mattern et al., 1996; Penman, 1995; Verheijen et al., 1986].

Major Internal Nuclear Matrix Proteins Are Present in All Four Different Human Cell Types

To determine (1) which proteins are common to all four cell types and which proteins are cell-type specific and (2) which proteins are part of the internal nuclear matrix, all 2-D gels containing nuclear matrix and nuclear shell preparations were matched and analyzed with the aid of the software package PDQUEST.
Proteins that are present in intact nuclear matrix preparations and are essentially absent in nuclear shells (i.e., less than 10% of the quantity in the matrix sample remains) are assumed to be putative internal matrix proteins [Mattern et al., 1996].

We determined which proteins in nuclear matrix or shell preparations, including the cytoskeletal proteins, were present in all four cell types. We found 44 common polypeptide spots after separation by IEF and 50 common proteins after NEPHGE (Fig. 5). For comparison, the total number of detected nuclear matrix proteins was at least 91 in IEF gels and at least 117 in NEPHGE gels. Note that several proteins are detectable in both IEF gels and NEPHGE gels. Also note that we counted identified proteins consisting of multiple spots as one. These proteins were identified previously by immunoblotting, comigration, microsequencing, and comparison with a 2D-gel protein database [Celis et al., 1994; Mattern et al., 1996].

Of each cell line, we quantitatively compared the protein composition of the nuclear matrix preparations with that of the nuclear shell preparations to determine the internal nuclear matrix proteins. For example, in the IEF gel in which 91 nuclear matrix proteins were de-
detected, 45 proteins fulfilled the criterion of internal nuclear matrix proteins. In the NEPHGE gel containing 117 nuclear matrix proteins, 53 scored as internal. Figure 5 shows all protein spots that we identified as internal nuclear matrix proteins common to all four cell lines (filled spots). Common proteins present in both nuclear matrices and nuclear shells are represented as open spots. Of the 44 common nuclear matrix proteins detected after IEF, 22 were internal nuclear matrix proteins. After NEPHGE, 30 of the 50 common proteins were identified as internal.

Evidently, more than half of the internal nuclear matrix proteins that we detected are present in all cell types. Of each cell type, we determined which proteins contribute more than 1% to the total amount of internal nuclear matrix protein as determined by silver staining. Table I shows the 25 internal nuclear matrix proteins that fulfill this criterion. Together, these proteins represent more than 75% of the total mass of internal nuclear matrix protein in all four cell types as estimated by silver staining. We identified 16 proteins as hnRNP proteins, one as the nucleolar protein B23, and one as the poly(A)-binding protein I (PABP I). The latter is probably not a true nuclear matrix protein because it is detected in the cytoplasm [Görlich et al., 1994]. All these abundant proteins were common to all four cell lines. We did not find any major internal nuclear matrix proteins to be cell-type specific. The only differ-
ences in the internal nuclear matrix protein composition between the cell types concerned very minor spots.

Quantitative Comparison of the Major Internal Nuclear Matrix Proteins of Different Human Cell Types

Because the protein composition of the internal nuclear matrix of the four cell types is qualitatively very similar, the next question was whether there are major quantitative differences between the cell types regarding these proteins. Table I shows the apparent relative quantities of the 25 most abundant internal nuclear matrix proteins, expressed as the percentage of the total amount of internal nuclear matrix protein as detected by silver staining. The proteins listed in Table I are arranged by order of quantity in HeLa S3 cells.

We found only small differences in relative quantity of most major internal nuclear matrix proteins among the four human cell lines. Of two unidentified proteins, numbered 1 and 3, the relative quantity was considerably higher in HeLa S3 cells than in the other cell types. Protein 3 migrates in the vicinity of hnRNP I, but it is not an isoform of hnRNP-I. Microsequencing of a peptide, obtained by partial diges-
tion of this protein, yielded the sequence VEEVKEEGPKEM, which has no homology with either hnRNP-I or any other known gene product. The relative quantity in the nuclear matrix of two other proteins was considerably higher in NT2/D1 than in the other cell types. One of these proteins is identified as hnRNP-A1, and the other protein (numbered 7) is unidentified. The quantity of the other internal nuclear matrix proteins did not differ appreciably across the cell types. Thus, the protein composition of the internal nuclear matrix is both qualitatively and quantitatively very similar in these four human cell types.

**DISCUSSION**

In this study, we investigated nuclear matrix preparations of four different human cell types to establish to what extent the protein composition of the internal nuclear matrix is the same in these cells types. Previously, we introduced a method to identify the internal proteins of the nuclear matrix [Mattern et al., 1996]. Internal nuclear matrix proteins can be distinguished from other proteins present in nuclear matrix preparations, i.e., proteins of the nuclear lamina and cytoskeletal proteins, by comparing 2-D gels containing nuclear matrix proteins with

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**Fig. 4.** Two-dimensional gel electrophoresis of nuclear matrices and nuclear shells of NT2/D1 cells. A–D as in Figure 1.
In this study, we used the same method to identify the major internal matrix proteins of four different human cell types and to examine to what extent these proteins are common to these cell types. Common internal matrix proteins likely fulfill a basal role in the organization of the nucleus.

In each cell type individually, we identified the major internal nuclear matrix proteins, defined as proteins that individually contribute more than 1% to the total protein mass of the internal nuclear matrix. The 25 proteins we classified as abundant in this way were present in all four human cell types. Together, these proteins represent more than 75% of the internal matrix protein mass in each cell type. This set of proteins mainly consists of the nucleolar protein B23 and the hnRNP proteins, which we identified previously as major internal nuclear matrix problems in HeLa S3 cells [Mattern et al., 1996]. B23 and some hnRNP proteins have also been detected in nuclear matrix preparations of other cell types and species, e.g., healthy rat liver [Nakayasu and Berezney, 1991]. This result indicates that the major internal nuclear matrix proteins of healthy cells are not different from those of tumor-derived cells.

We found only minor differences in the relative amounts of most major internal nuclear matrix proteins among the cell types we examined. Only the quantity of four proteins was considerably higher in one cell type compared with the other three cell types, one of which was hnRNP-A1. We cannot decide from these experiments whether the differences in quantity are due to differences in relative amounts in the cell or to differences in association with the nuclear matrices in the different cell types. Most of the hnRNP-A1 protein is extracted from the nuclear matrix of HeLa S3 cells [Mattern et al., 1996]. Thus, hnRNP-A1 may be more tightly associated to the nuclear matrix of NT2/D1 cells.

Remarkably, we did not find major internal nuclear matrix proteins that are cell-type specific. We did find some differences in the internal nuclear matrix protein composition across the cell types, but these differences concerned only minor proteins. Several investigators have reported the presence of cell-type-specific proteins in nuclear matrix preparations of tumor-derived and healthy cells [Bidwell et al., 1994; Dworetzky et al., 1990; Fey and Penman, 1988; Getzenberg et al., 1996; Kallajoki and Osborn, 1994; Keesee et al., 1994; Khanuja et al., 1993;
Evidently, these cell-type-specific nuclear matrix proteins are of low abundance. In these studies, the difference on 2-D gels between minor and major proteins is often not very clear due to overloading of the gels. In contrast, in our study, we were very careful when loading gels so that staining of all protein spots was in the linear range. Also, some of these cell-type-specific proteins may in fact be cytoskeletal proteins and not nuclear matrix proteins.

The most striking differences in protein composition of nuclear matrices isolated from different cell types were not internal nuclear matrix proteins but rather proteins of the cytoskeleton, particularly intermediate filament proteins. Different human cell types contain different keratins [Moll et al., 1982]. Heuijerjans et al. [1989], for instance, showed the absence of vimentin in T24 cells. Methods have been developed to deplete the intermediate filament proteins from nuclear matrix preparations, for example, by solubilizing the nuclear matrix preparations in 8M urea followed by dialysis and centrifugation [Fey and Penman, 1988] or by sheering nuclei by forcing these through a fine needle [Belgrader et al., 1991]. However, such preparations still contain intermediate filament proteins and other cytoplasmatic proteins [Kallajoki and Osborn, 1994; Mattern et al., 1996]. Although PABP I seemed to fulfill our criterion for a major internal nuclear matrix protein, its presence in nuclear matrix preparations is probably due to association with the cytoskeleton. PABP I is localized in the cytoplasm, as shown by confocal immunofluorescence microscopy [Görlach et al., 1994]. PABP I probably remains attached to the cytoskeleton during nuclear matrix isolation unless RNase is used to dissociate the internal nuclear matrix. However, at least 17 of the other 24 abundant internal matrix proteins were identified as genuine nuclear proteins, i.e., hnRNP proteins and B23.

We conclude that the fibrogranular internal nuclear matrix exists mainly of hnRNP proteins. The hnRNP proteins are conserved in many species [Dreyfuss et al., 1993], which fortifies the suggestion that these proteins play an essential role in the nucleus. The exact function of hnRNP proteins is not yet clear, but they are probably involved in many aspects of RNA metabolism [Dreyfuss et al., 1993; Kiledjian et al., 1994]. These proteins are defined as hnRNA (including pre-mRNA) binding proteins, which are not stable components of other nuclear structures such as small nuclear ribonucleoproteins. Most hnRNA is retained in nuclear matrix preparations [see He et al., 1990; Wansink et al., 1996]. Therefore, it is likely that the internal fibrogranular structure of the internal matrix consists mainly of hnRNP complexes. In this view, the fibrogranular structure of the internal nuclear matrix is dedicated mainly to the synthesis, processing, packaging, and transport of hnRNA. Therefore, this component of the nuclear matrix is likely the result of transcription. However, there are also indications

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aSpot indications correspond to Figure 5 (h, hnRNP; PI, PABPI).
bQuantity is expressed as percentage of the total amount of internal nuclear matrix protein after silver staining. Numbers are the mean of two experiments in which each preparation was run in duplicate on two-dimensional gels. The relative average range (difference between the two values as a percentage of the average value) is 26.9% (±19.3% S.D.).
cThe quantity of these proteins may be underestimated due to streaking.

Partin et al., 1993; Stuurman et al., 1989]. Evidently, these cell-type-specific nuclear matrix proteins are of low abundance. In these studies, the difference on 2-D gels between minor and major proteins is often not very clear due to overloading of the gels. In contrast, in our study, we were very careful when loading gels so that staining of all protein spots was in the linear range. Also, some of these cell-type-specific proteins may in fact be cytoskeletal proteins and not nuclear matrix proteins.
that the nuclear matrix plays a more active role in chromatin organization and gene expression. Some hnRNP proteins are capable of DNA binding. For instance, hnRNP-U (SAF-A) binds S/MARs [Romig et al., 1992; Von Kries et al., 1994], and hnRNP-K binds and transactivates the c-myc promoter [Tonomaga and Levens, 1995]. The internal matrix may be formed by a highly dynamic framework of mainly hnRNPs that embeds the interphase chromatin fibers and is connected to the chromatin via the nascent hnRNA chains and/or directly via DNA-binding hnRNP proteins [De Jong et al., 1996].

Protein–protein, protein–RNA, and protein–DNA interactions in the nuclear matrix may play a crucial role in bringing together chromatin, transcription complexes, and the RNA processing and packaging machinery in the correct spatial arrangement. This notion is in agreement with the idea that the internal nuclear matrix is involved in functionally and spatially organizing the interphase nucleus.

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