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hnRNP Proteins and B23 Are the Major Proteins of the Internal Nuclear Matrix of HeLa S3 Cells

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Abstract The nuclear matrix is the structure that persists after removal of chromatin and loosely bound components from the nucleus. It consists of a peripheral lamina–pore complex and an intricate internal fibrogranular structure. Little is known about the molecular structure of this proteinaceous internal network. Our aim is to identify the major proteins of the internal nuclear matrix of HeLa S3 cells. To this end, a cell fraction containing the internal fibrogranular structure was compared with one from which this structure had been selectively dissociated. Protein compositions were quantitatively analyzed after high-resolution two-dimensional gel electrophoresis. We have identified the 21 most abundant polypeptides that are present exclusively in the internal nuclear matrix. Sixteen of these proteins are heterogeneous nuclear ribonucleoprotein (hnRNP) proteins. B23 (numatrin) is another abundant protein of the internal nuclear matrix. Our results show that most of the quantitatively major polypeptides of the internal nuclear matrix are proteins involved in RNA metabolism, including packaging and transport of RNA. © 1996 Wiley-Liss, Inc.

Key words: nuclear matrix, HeLa S3 cells, 2-D gel electrophoresis, heterogeneous nuclear ribonucleoproteins, B23

The nuclear matrix (nucleoskeleton, nuclear scaffold) is an operationally defined structure, that persists after extraction of nuclei with detergents, nucleases, and often high-ionic-strength buffers. This structure is thought to play an important role in several nuclear functions, such as organization and replication of the genome [Cook, 1991; De Jong et al., 1990; Getzenberg et al., 1991; Jackson and Cook, 1986; Mirkovitch et al., 1984; Nakayasu and Berezney, 1989], transcription [Jackson and Cook, 1985; Razin and Yarovaya, 1985; Stein et al., 1991; Stein et al., 1994; van Steensel et al., 1995; Wansink et al., 1996] and RNA processing [Blencowe et al., 1994; Zeitlin et al., 1989; Zeng et al., 1994a]. Although it is more than 20 years ago since the nuclear matrix was first described [Berezney and Coffey, 1974], the molecular structure and the precise function of the internal nuclear fibrogranular network are unclear.

The nuclear matrix can be divided into two substructures: (1) the lamina pore complex, and (2) the internal matrix, consisting of a fibrogranular structure and containing residual nucleolar structures. Detailed information is available about the structural proteins of the lamina-pore complex, the lamins [reviewed by Dessev, 1990; Georgatos et al., 1994; Gerace and Burke, 1988; Hutchison et al., 1994; Nigg, 1989]. Much less is known about the composition and structure of the internal nuclear matrix [for reviews, see Fey et al., 1991; Mattern et al., 1996; Stuurman et al., 1992a; Verheijen et al., 1988]. In the past two decades, many investigators have studied the protein composition of nuclear matrices [e.g., Fey et al., 1986; Kallajoki and Osborn, 1994; Kaufmann and Shaper, 1984; Nakayasu and Berezney, 1991; Stuurman et al., 1990; Verheijen et al., 1986]. Although the apparent ultrastructure and the precise protein composition of the nuclear matrix to some extent depend on the isolation procedure and cell type, many proteins of the nuclear matrix are common for most or all cell types [Mattern et al., 1996; Stuurman et al., 1992a]. Many components of the nuclear machineries for transcription, RNA processing, and replication are tightly associated with this structure (see references cited above). However, no systematic analysis

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has been carried out to determine which proteins are the quantitatively major components of the internal matrix. The answer to this question may shed new light on the structure and function of the nuclear matrix.

Evidence has been presented that the internal nuclear matrix contains filamentous structural proteins, some of which may be related to those of the cytoskeleton. Several observations support this view: (1) filamentous actin has been found in neuronal nuclei [Amankwah and Deboni, 1994], (2) intermediate filament-like structures have been observed in the nuclear matrix [He et al., 1990; Jackson and Cook, 1988], and (3) antibodies against the intermediate filament type protein lamin A [Hozák et al., 1995] and against the coiled coil protein NuMA [Zeng et al., *1994b*] have been shown to decorate at least some of the nuclear matrix fibres. Evidently, these and possibly other proteins that are able to form filamentous structures are present in the internal nuclear matrix. In addition, a structural role for RNA is suggested based on the fact that the structure of the nuclear matrix is affected by treatment with RNase [Belgrader et al., 1991; Fey et al., 1986; He et al., 1990]. In agreement with this view are reports on the presence of hnRNP proteins in the nuclear matrix [e.g., Fey et al., 1986; Verheijen et al., 1986].

The aim of this paper is to identify the quantitatively major proteins of the internal nuclear matrix of HeLa S3 cells. To this end, the protein composition of a cell fraction that contains the internal nuclear fibrogranular structure was compared with a cell fraction from which this structure had been selectively dissociated. Systematic analysis of two-dimensional gels resulted in the identification of the 21 most abundant internal nuclear matrix proteins, including B23 and 16 hnRNP proteins. These proteins together represent about 75% of the total amount of internal nuclear matrix protein. Our results show that the major internal matrix proteins are involved in RNA metabolism.

MATERIALS AND METHODS

Cell Culture

HeLa S3 (human cervix carcinoma) cells were grown as suspension culture in roller bottles at 37°C in 10% CO_2-saturated Joklik's modified minimum essential medium (Gibco, Paisly, UK), supplemented with 5% (v/v) heat-inactivated fetal calf serum (Gibco), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 µl/ml streptomycin.

Isolation of Nuclear Matrices and Nuclear Shells

Nuclear matrices were isolated as described by [de Graaf et al., 1992] with some modifications. All incubations were carried out at 0–4°C at a cell density of 5×10^7 cells/ml, unless stated otherwise. 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 $MgCl_2$, 5 U/ml RNasin (Promega, Madison, WI), 1 mM EGTA, 1 mM PMSF, 1 µg/ml leupeptin), containing 1% (w/v) Triton X-100 plus extra 15 U/ml RNasin. The lysate was subsequently passed 5 times through a 22-gauge needle [Belgrader et al., 1991]. After centrifugation for 5 min at 400*g* nuclei were incubated for 30 min in CSK100 buffer, containing 0.5 mM sodium tetrathionaat for stabilization of the internal nuclear matrix [Kaufmann et al., 1981; Stuurman et al., 1992b]. Nuclei were washed twice with CSK50 buffer (as CSK100 buffer; however, 50 mM NaCl instead of 100 mM) by centrifugation and were then digested at a density of 2×10^8 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 matrices were pelleted by centrifugation at 1,000*g* for 5 min and washed once with CSK50.

Nuclear shells, which are cell fractions without the internal matrix structure [Ludérus et al., 1992], were isolated like nuclear matrices with the following modifications. RNasin and sodium tetrathionate were omitted. Instead, 1 mM dithiothreitol (DTT) was added to all buffers. Additionally, matrices thus obtained were digested with 50 µg/ml RNase A at a density of 1×10^8 matrices/ml in CSK50 buffer for 15 min at 25°C. Subsequently, matrices were extracted for 15 min by addition of NaCl to a final concentration of 2 M and DTT to a final concentration of 40 mM. Nuclear shells were collected by centrifugation at 14,000*g* for 20 min and washed once with CSK50.

Electron Microscopy

Suspensions of nuclear matrices and nuclear shells in CSK50 were spotted onto Thermanox (Miles Lab, Naperville, IL) coated with Alcian

Blue and were allowed to attach for at least 30 min. Structures were then fixed with 1% glutaraldehyde in CSK50 without leupeptin and RNasin. Preparations were cryoprotected for 30 min with 30% (v/v) N,N-dimethylformamide in PBS [Meissner and Schwarz, 1990], frozen in liquid propane in a KF80 rapid-freeze apparatus [Reichert-Jung, Wien, Austria], and freeze-substituted with 0.5% (w/v) uranylacetate in methanol [Humbel and Müller, 1986]. Subsequently, preparations were embedded in Epon and ultrathin sections were cut parallel to the substratum. The sections were post-stained with 2% (w/v) uranyl acetate and 0.4% (w/v) lead citrate [Venable and Coggeshall, 1965] for 10 min each and examined in a Philips EM 420 electron microscope at 120 kV.

Immunopurification of hnRNP Complexes

hnRNP complexes were immunopurified essentially as described by [Piñol-Roma et al., 1990]. Briefly, HeLa S3 nuclei were disrupted by sonication in immunopurification buffer (10 mM Tris–HCl, pH 7.4, 100 mM NaCl, 2.5 mM $MgCl_2$, 0.5% (w/v) Triton X-100, 5 μg/ml aprotonin and 2 μg/ml each of leupeptin and pepstatin). The nucleoplasm was collected after centrifugation on a 30% sucrose cushion. This fraction was incubated for 10 min with the hnRNP-C1/C2 antibody 4F4, that had been coupled to protein A Sepharose beads using dimethylpimelidate as described by Harlow and Lane [1988]. The beads, now containing hnRNP complexes, were finally washed 6 times with the immunopurification buffer.

Western Blotting

Proteins were separated by one-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) [Laemmli, 1970] or two-dimensional gel electrophoresis (see below) and blotted onto nitrocellulose [Towbin et al., 1979], with the addition of 0.1% SDS to the transferbuffer. Blots were immunostained as described by van Steensel et al. [1995], using the following monoclonal antibodies: 41CC4 against lamins A and C [Burke et al., 1983], 4F4 against hnRNP-C1/C2 [Choi and Dreyfuss, 1984], 4D11 against hnRNP-L [Piñol-Roma et al., 1989], 4B10 against hnRNP-A_1 [Piñol-Roma et al., 1988], 7G12 against hnRNP-I [Ghetti et al., 1992], 10E10 against PABP I [Görlach et al., 1994], F1 against NuMA [Compton et al., 1992], anti-actin (N350, Amersham, Buckinghamshire, UK), RV202 against vimentin, RCK106 against keratin 18, and RCK105 against keratin 7 [Ramaekers, 1987]. We also used polyclonal antibodies against SAF-A/hnRNP-U [Fackelmayer et al., 1994], against PAB II [Krause et al., 1994], and against B23 [Wang et al., 1993].

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. 1% (w/v) CHAPS was added to 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 (0.5% pH 3–10, 0.5% pH 5–8, and 1% pH 7–9; BioRad) and were run for 1 h at 200 V and 4 h at 700 V. For the second dimension, 10% SDS-polyacrylamide gels were used. Gels were silver stained using the method of Heukeshoven and Dernick [1986], as described by Rabilloud [1992] with some modifications. Gels were fixed overnight in 40% (v/v) ethanol and 10% (v/v) acetic acid, incubated in sensitizer (30% (v/v) ethanol, 0.5% glutaraldehyde, 0.5 M sodium acetate, 2 g/L sodium thiosulfate pentahydrate) for 2 h, and washed 3 times for 20 min in deionized water. After impregnation of the gels in 2 g/L silver nitrate plus 0.25 ml/L of a 37% formaldehyde solution for 1 h, the gels were washed for 20 s in water and developed for 10 min in 60 g/L sodium carbonate, 0.15 ml/L of 37% formaldehyde, and 10 mg/L sodium thiosulfate pentahydrate. The reaction was stopped by adding 50 g/L Tris and 2% (v/v) acetic acid. The gels were scanned using a Molecular Dynamics (Kent, UK) laser scanner. PDQUEST software (PDI, New York, NY) was used for the quantitative analysis of the gels. Standards for 2-D SDS–PAGE (BioRad) were used for determination of M_r and pI. Also, several samples were run precisely as described by Celis et al. [1993]; results were compared to the human keratinocyte two-dimensional gel protein database [Celis et al., 1994]. hnRNP proteins were separated by NEPHGE, using 2% ampholytes pH 3–10, in the first dimension. For the second dimension, 12% SDS-PA gels were used.

Microsequencing

Spots from several Coomassie Blue stained 2-D gels were pooled and loaded on a 15% SDS-polyacrylamide gel together with 20 ng V8 protease (Promega). The protein was digested for 30 min after migration into the stacking gel [Cleveland et al., 1977]. Peptides thus obtained were separated by electrophoresis and electroblotted onto PVDF membrane (Immobilon-PSQ, Millipore, Bedford, MA) using 50 mM Tris and 50 mM boric acid as transfer buffer. The peptides were visualized by staining with 0.1% Coomassie Blue in 50% methanol for 2 min and sestaining with 50% methanol. Amino acid sequences were determined with a Procise 494 Protein Sequencer (Perkin-Elmer, Applied Biosystems division, Foster City, CA). The identity of the proteins was determined by submitting the obtained partial sequence to the BLITZ server at the European Bioinformatics Institute (EBI, UK).

RESULTS

Criterium for Identification of Internal Nuclear Matrix Proteins

The first step in the identification of proteins of the internal nuclear matrix is to formulate a criterium to recognize internal matrix proteins. To this end, we compared quantitatively the protein composition of a nuclear fraction containing the internal fibrogranular matrix with a nuclear fraction lacking this internal structure. We refer to these two fractions as nuclear matrices and nuclear shells, respectively [Ludérus et al., 1992]. Proteins present in the first structure and completely lacking in the latter are putative internal matrix proteins.

In order to prepare intact nuclear matrices, isolated nuclei from HeLa S3 cells [Belgrader et al., 1991] were treated with a mild oxidizing agent, 0.5 mM sodium tetrathionate. This agent has been shown to stabilize the internal nuclear matrix structure [Belgrader et al., 1991; Kaufmann et al., 1981; Neri et al., 1995; Stuurman et al., 1992b]. Subsequently, nuclear matrices were prepared from nuclei by DNase I digestion (RNase free), followed by 0.25 M ammonium sulfate extraction [Fey et al., 1986] to remove chromatin and loosely bound nuclear components. We used the RNase inhibitor RNasin to prevent degradation of nuclear RNA, which may have a structural function in the nuclear matrix [He et al., 1990]. Nuclear shells were prepared from nuclear matrices that were not stabilized by sodium tetrathionate, by dissociation of the internal matrix under reducing conditions, i.e., in the presence of dithiothreitol [Belgrader et al., 1991; Kaufmann and Shaper, 1984; de Graaf et al., 1992; Stuurman et al., 1990] and by addition of RNase [Belgrader et al., 1991; Fey et al., 1986].

The criterium for identification of internal matrix proteins that we have formulated above depends on the notion that the internal matrix is present in nuclear matrix preparations isolated under mild oxidizing conditions and is completely dissociated under conditions we employ for isolation of nuclear shells. To check this, nuclear matrices (Fig. 1A) and nuclear shells (Fig. 1B) were examined by electron microscopy. The internal matrix, consisting of residual nucleolar structures embedded in a fibrogranular network, is clearly visible in the nuclear matrices. The nuclear shells, as expected, were devoid of this internal structure. Note that a considerable amount of residual cytoskeleton is present in both preparations, reflecting the tight association of the nuclear matrix and the cytoskeleton [Capco et al., 1982; Kallajoki and Osborn, 1994].

Protein Composition of Nuclear Matrices Versus Nuclear Shells

The protein composition of nuclear matrices and nuclear shells was determined by two-dimensional gel electrophoresis (Fig. 2). Both IEF and NEPHGE were used as a first dimension to be able to separate acidic as well as basic proteins. For the second dimension we employed SDS–PAGE. Figure 2 shows that the protein composition of nuclear matrices is clearly different from that of nuclear shells. Polypeptides present in nuclear matrices and absent in nuclear shells are likely to be components of the internal nuclear matrix. Subsequently, these proteins were quantitatively analyzed and further characterized.

Considerations Concerning Quantitative Analysis of Two-Dimensional Gels

For a reliable quantitative analysis, staining of the proteins has to be linear in relation to the amount of protein and should be reproducible. We choose the silver staining procedure of Heukeshoven and Dernick [1986] as described by Rabilloud [1992]. The linearity and reproducibility of the silver staining method was tested by running four two-dimensional gels in duplicate,

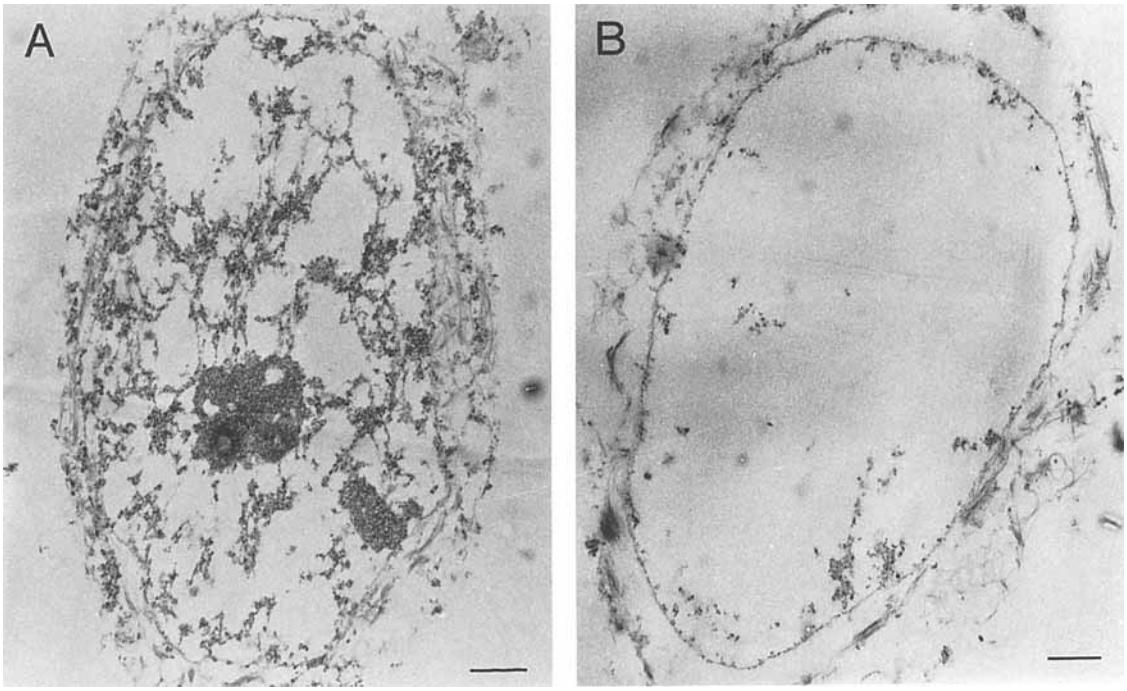

Fig. 1. Ultrastructure of nuclear matrices and nuclear shells. Electron microscopy images of thin sections of nuclear matrices (**A**) and nuclear shells (**B**) isolated from HeLa S3 cells. Scale bar = 1 μm.

each of the four gels containing a different total amount of nuclear matrix proteins. All gels were silver stained under identical conditions. For 18 randomly selected proteins, the integrated optical density (OD) values were determined with the aid of PDQUEST software. Figure 3 shows for three of the 18 proteins that the OD linearly increased with increasing amounts of protein, up to a certain amount of nuclear matrix equivalents applied to the gel. For example, for lamin B1, staining was linear up to 2.0×10^6 nuclear equivalents applied per gel. Up to 1.0×10^6 nuclear equivalents, the staining of all 18 proteins that we tested was linear. At higher protein loads, staining of abundant proteins showed saturation. Therefore, we applied in all further experiments 1.0×10^6 nuclear equivalents. Also note that, if this amount is applied, the reproducibility is very good. These results show that the silver staining method can be used in our quantitative analysis.

To compensate for small gel-to-gel variation, e.g., due to errors in application of protein samples, we have normalized OD values of each gel on the basis of protein markers. For this, proteins have been chosen that are present in the same amount in matrices and in shells. Lamins are expected to be present in both preparations, because lamins are structural proteins of the lamina. However, Figure 4 shows that lamins A and C are partially extracted from the nuclear lamina during isolation of shells. Therefore, these two proteins cannot be used for normalization. Lamin B1 and vimentin, the latter being part of the adhering cytoskeletal structure, were quantitatively retained by the shells (Fig. 4). These two proteins have been used as internal standards for normalization of IEF gels. Lamin B1 and vimentin were not resolved well by NEPHGE. Therefore, we have selected another protein for normalization on these gels. We choose a spot, marked with an asterisk (*) in Figure 5, that was well resolved by both IEF and NEPHGE, and was present in the same quantity in matrices and shells after normalization of the IEF gels on the basis of vimentin or lamin B1.

Identification of Internal Nuclear Matrix Proteins by Quantitative Analysis of Two-Dimensional Gels

Silver-stained 2-D gels containing proteins of the two nuclear fractions and run in duplicate, were quantitatively analyzed, matched and compared by using PDQUEST software. With this method, 106 proteins could be detected in gels of

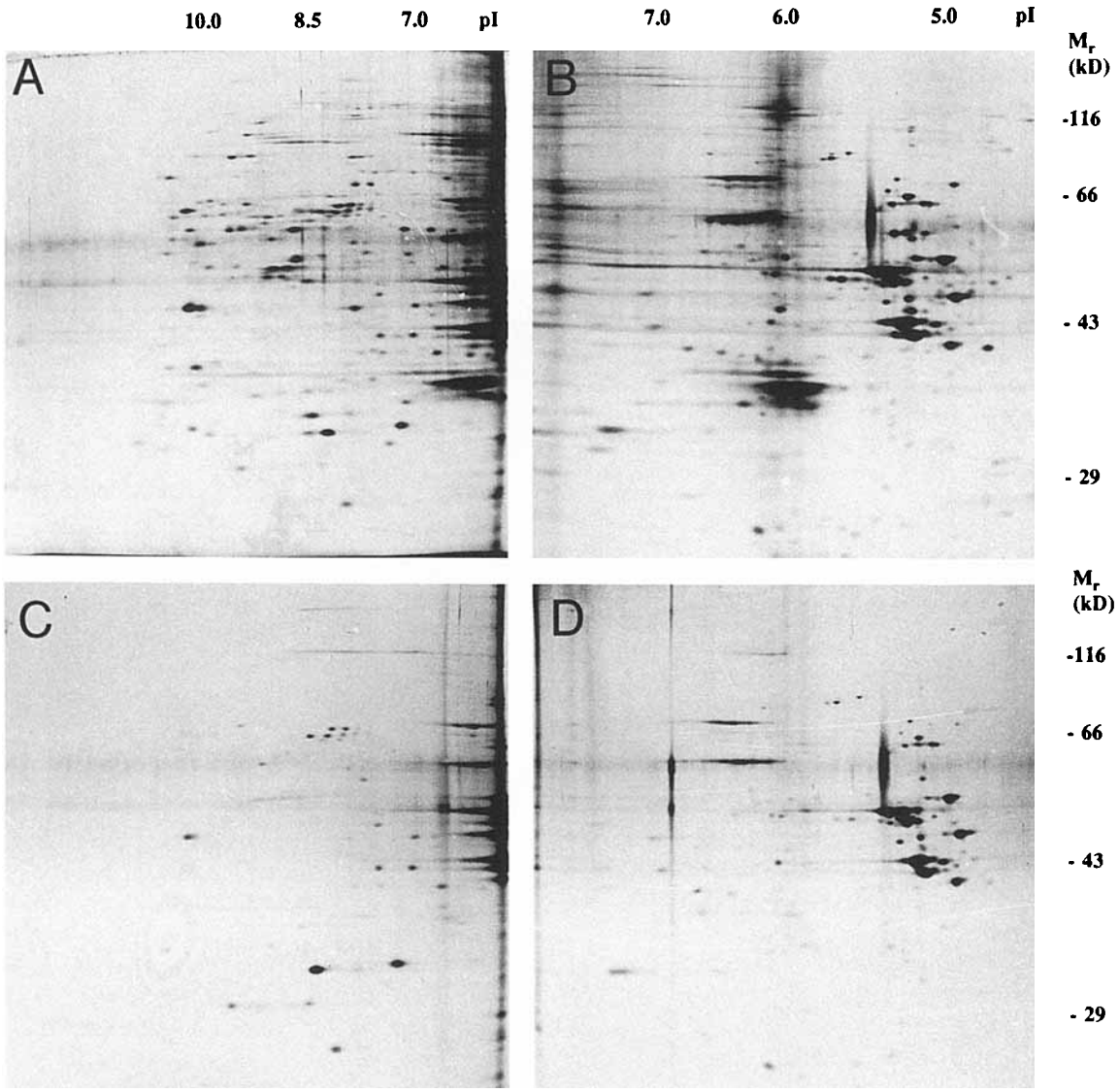

Fig. 2. Two-dimensional gel electrophoresis of nuclear matrices and nuclear shells. Proteins of nuclear matrices (**A, B**) and nuclear shells (**C, D**) were separated by NEPHGE/SDS–PAGE (**A, C**) and IEF/SDS-PAGE (**B, D**). Each gel contains proteins from 10^6 nuclear equivalents. Proteins were detected by silver staining.

nuclear matrices after IEF, and 91 proteins after NEPHGE. In gels of nuclear shell preparations 50 and 27 proteins were detected with IEF and NEPHGE, respectively. Next, we defined a threshold to decide which proteins are present exclusively in the internal matrix and which are not. We stipulated that if the quantity of a protein in the shell sample is less than 10% of the quantity of that protein in the matrix sample, it is probably an internal matrix protein.

Proteins identified as internal matrix proteins by the criterium formulated above are indicated by filled spots in Figure 5. Proteins present in both nuclear shells and nuclear matrices are represented by open spots. Of the 106 nuclear matrix proteins detected on the IEF gels, 56 were putative internal matrix proteins. Of the 91 proteins detected after NEPHGE, 64 were putative internal matrix proteins. If we changed the 10% threshold to 5% or to 20%, we obtained the same result. Evidently, a well defined set of proteins exists that is lost under conditions that result in selective dissociation of the internal, fibrogranular nuclear matrix.

Most of the proteins that we identified as putative internal matrix proteins on 2-D gels after IEF or after NEPHGE, were present only in minute amounts and were barely visible after

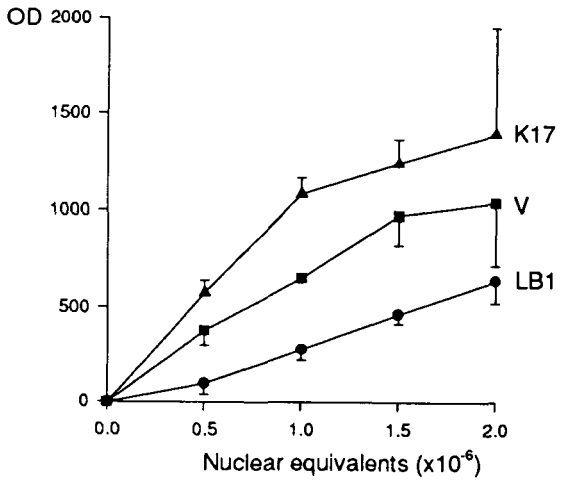

Fig. 3. Linearity of silver staining. Different amounts of nuclear matrices were separated by 2-D gel electrophoresis (IEF/SDS–PAGE) in duplicate. Each gel was silver stained under the same conditions. The integrated OD of 18 spots was calculated with the aid of PDQUEST software. Shown are the OD values (mean ± SD) of three spots (LB1, lamin B1; K17, keratin 17; V, vimentin) as a function of the amount of nuclear matrix proteins (nuclear equivalents) that was applied to the gel.

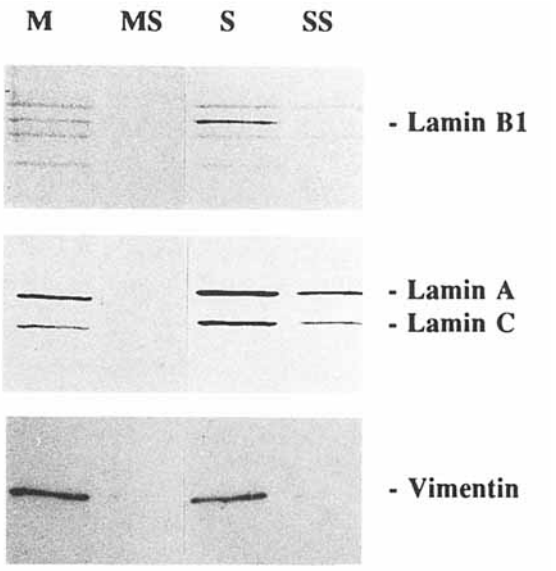

Fig. 4. Retention of lamins and vimentin by nuclear matrices and shells. Proteins of nuclear subfractions were separated by SDS–PAGE and transferred to nitrocellulose. The blots were immunostained with antibodies against lamin B1, lamins A/C and vimentin. Each lane contains protein from 10^6 nuclear equivalents. The lanes contain nuclear matrices (M), proteins extracted by DNase digestion and 0.25 M ammonium sulfate (MS), nuclear shells (S), and proteins extracted by RNase digestion, 40 mM DTT, and 2 M NaCl (SS).

silver staining. One should note here that the OD per μg protein after silver staining differs from protein to protein [Gersten et al., 1991]. Therefore, an absolute comparison of amounts of proteins is not possible. For that reason we also used Coomassie Blue staining. Table I lists the 21 most abundant putative internal matrix proteins, as judged by both silver and Coomassie Blue staining. Together, these proteins represent about 75% of the total amount of internal matrix protein, expressed in OD units after silver staining. For comparison, each of these proteins is about equal or more abundant than lamin B1, the least abundant of the three major lamin proteins.

Characterization of Internal Nuclear Matrix Proteins

Figure 5 and Table I indicate which proteins have been identified by immunoblotting, by comigration, by microsequencing and/or by comparison with the protein database of Celis et al. [1994]. The most abundant proteins in our preparations are cytoskeletal proteins (i.e., actin, vimentin, and keratins), identified by immunoblotting. These proteins were present in the same amounts in matrix preparations and in shells. This is in agreement with observations of others who showed that the nuclear matrix in tightly associated with the intermediate filament system [Capco et al., 1982; Penman, 1995; Verheijen et al., 1986]. Of these cytoskeletal proteins, only a small amount (less than 10%) of actin and of keratin 18 dissociate from the preparations under conditions that removes the internal matrix.

Previously, hnRNP proteins have been shown to be associated to the nuclear matrix [e.g., Fey et al., 1986; Verheijen et al., 1986; reviewed by Mattern et al., 1996; Verheijen et al., 1988]. Among the 21 most abundant internal nuclear matrix proteins, we identified 16 hnRNP proteins (Fig. 5 and Table I). We used the nomenclature of [Piñol-Roma et al., 1988]. These proteins were identified by comigration of hnRNP proteins, immunopurified according to Piñol-Roma et al. [1990], with nuclear matrix proteins on 2-D gels (Fig. 6). The identification of some hnRNP proteins was confirmed by immunoblotting, by comparison with the 2-D gel protein database [Celis et al., 1994] and/or by microsequencing (Table I). Amino acid sequences were obtained after partial digestion of the proteins with V8 protease. This resulted in the identification of hnRNP-A2 (sequence YGKIDTI, corresponding to residues 135–141) and hnRNP-K (sequence GLQLPSPT, corresponding to resi-

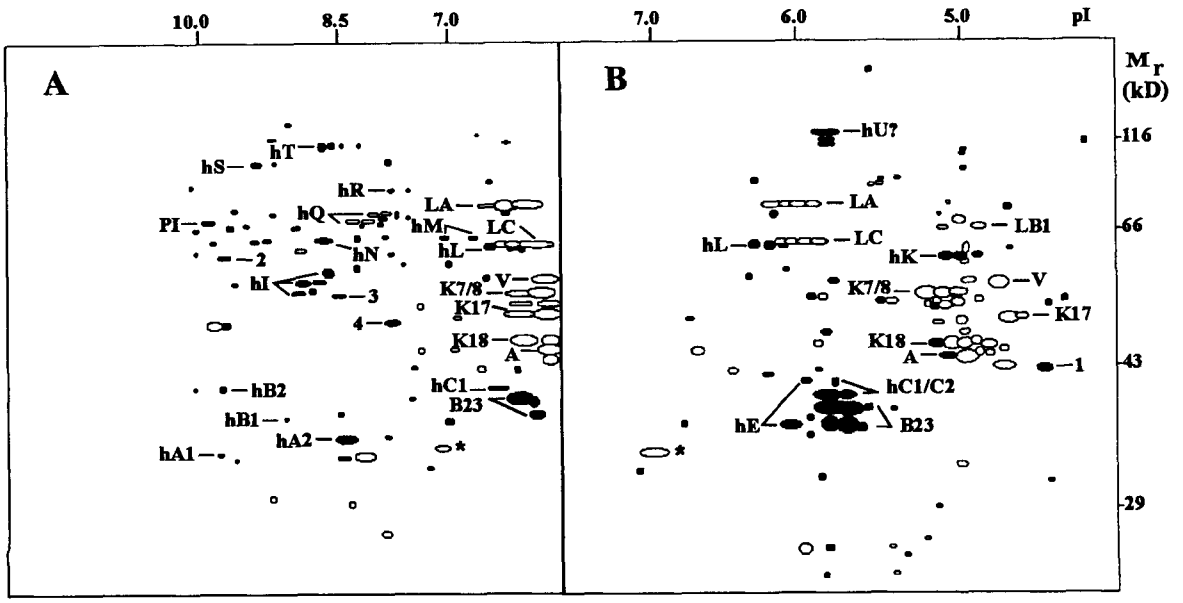

Fig. 5. Identification of peripheral and internal matrix proteins. Schematic representation of 2-D gels (A: NEPHGE; B: IEF) containing nuclear matrix proteins. Proteins that were identified as internal proteins by quantitative analysis (see text) are represented as filled spots (h, hnRNP; B23; PI, poly(A)-binding protein I); proteins present in nuclear shell preparations are represented as open spots (V, vimentin; A, actin; K, keratin; L, lamin). Major internal matrix proteins that have not been identified yet are numbered (see also Table I). The spot used for normalization of NEPHGE gels is labeled with an asterisk (*). Note that the area of spots does not necessarily reflect the relative amount of protein.

dues 111–118). hnRNP-Q was also present in nuclear matrix preparations. However, more than 20% of hnRNP-Q remained associated to the nuclear shells. Although some hnRNP-A1 was present in the internal nuclear matrix, most of this protein was not retained by the nuclear matrix under our conditions, as shown by immunoblotting (Fig. 7). SAF-A (scaffold attachment factor A), recently identified as hnRNP-U [Fackelmayer et al., 1994], is an abundant nuclear protein and a constituent of the nuclear matrix [Romig et al., 1992]. We were not certain about the position of this protein on two-dimensional gels, because it did not migrate well in the first dimension gel. Therefore, we have also analyzed nuclear subfractions by one-dimensional SDS–PAGE. Detection of hnRNP-U/SAF-A by immunoblotting showed that 50% is retained by the nuclear matrix and that the protein is completely absent form nuclear shell preparations, like hnRNP-C1/C2 (Fig. 7). Therefore, also hnRNP-U can be considered as an (abundant) internal matrix protein.

We also found another RNA-binding protein among the abundant internal nuclear matrix proteins: poly(A)-binding protein I (PABP I, see Table I and Fig. 5). This protein could be identified by the partial amino acid sequences LYEKF (corresponding to residues 27–31) and EAAERA (corresponding to residues 149–154), which we obtained by microsequencing. Its identity was confirmed by immunoblotting and by comparison with the 2-D gel protein database [Celis et al., 1994]. Interestingly, we found that hnRNP-P comigrated with PABP I (Fig. 6). hnRNP-P is indeed able to bind poly(A) [Swanson and Dreyfuss, 1988] and therefore it is probably the same protein as PABP I. PABP I is, however, found to be localized to the cytoplasm [Görlach et al., 1994]. It is possible that it is not a nuclear matrix protein, but is associated to the cytoskeleton. PAB II is a nuclear poly(A)-binding protein, which is distinct from PABP I by its M_r of 49 kD and amino acid sequence [Wahle, 1993]. We could not identify PAB II unambiguously on two-dimensional gels, so we detected the protein by immunoblotting after one-dimensional SDS–PAGE of nuclear subfractions (Fig. 7). Like hnRNP-C1/C2 and hnRNP-U/SAF-A, the PAB II protein is retained by the nuclear matrix for about 50% and is completely absent in nuclear shells. Therefore, also PAB II can be considered as an internal matrix protein.

We found that also B23 (numatrin, nucleophosmin) is a very abundant internal nuclear matrix protein (Fig. 5; Table I). Previously, this

TABLE I. Major Internal Nuclear Matrix Proteins

Spot no.[a]	pI (pH)	M_r (kD)	Quantity[b]	Identification[c]	Method[d]
IEF					
B23	5.8–5.9	34.0–36.0	19.1 (7.9)	B23 (numatrin)	D, I
hC1/C2	5.8–5.9	37.2–37.6	8.6 (n.d.)	hnRNP-C1/C2	C, D, I
hE	6.0–6.1	34.1–39.5	2.7 (4.0)	hnRNP-E	C
hK	5.2–5.4	59.7	4.4 (2.4)	hnRNP-K	C, M
hL	6.3–6.4	62.5	1.8 (3.6)	hnRNP-L	C, I
hU (?)	5.8–5.9	125.9	1.4 (3.1)	hnRNP-U (SAF-A)	C
1	4.5	41.4	2.4 (0.8)		
NEPHGE					
hA1	9.9	33.1	0.6 (6.8)	hnRNP-A1	C, D
hA2	8.6	33.9	2.9 (19.4)	hnRNP-A2	C, D, M
hB1	9.1	35.0	0.6 (2.4)	hnRNP-B1	C, D
hB2	9.9	37.5	1.1 (5.7)	hnRNP-B2	C, D
hI	8.8–9.0	55.4–57.4	6.1 (9.5)	hnRNP-I (PTB)	C, I
hM	6.6–7.0	64.9	1.2 (1.5)	hnRNP-M	C
hN	8.8	64.2	2.7 (1.8)	hnRNP-N	C
hR	7.7–8.0	82.2	0.5 (2.3)	hnRNP-R	C
hS	9.2–9.4	97.0	1.0 (6.5)	hnRNP-S	C
hT	8.5–8.8	108.1	1.2 (2.4)	hnRNP-T	C
PI	10.0	69.4	1.0 (3.7)	PABP I (hnRNP-P)	C, D, I, M
2	9.9	60.4	1.1 (1.8)	—	—
3	8.7	53.0	1.2 (2.8)	—	—
4	8.0	47.5	1.7 (1.6)	—	—

[a]Spot indications correspond with Figure 5. The position of hnRNP-U is not certain.
[b]Quantity is expressed relative to the quantity of lamin B1 after silver staining and, between brackets, after Coomassie Blue staining. The quantity of some proteins may be underestimated, due to streaking (n.d. = not determined).
[c]hnRNP, heterogeneous nuclear ribonucleoprotein; SAF-A, scaffold attachment factor A; PTB, polypyrimidine tract binding protein; PABP I, poly(A)-binding protein I.
[d]Methods of identification: C: comigration on 2-D gel; D: comparison with 2-D gel protein database [Celis et al., 1994]; I, immunoblotting; M, microsequencing.

protein was found to be associated to the rat liver nuclear matrix [Fields et al., 1986; Nakayasu and Berezney, 1991]. B23 is a nucleolar protein. However, immunolabeling studies have shown that it is also present throughout the nucleoplasm, but at lower concentration (J.M. Bridger, M.E. Kerr, and C.J. Hutchison, personal communication). It is unknown whether B23 is part of the internal fibrogranular matrix.

NuMA (nuclear mitotic apparatus protein) is a nuclear matrix protein [Kallajoki et al., 1991; Lydersen and Pettijohn, 1980]. Considering its abundancy, 2×10^5 copies per cell, and its putative ability to form coiled coil structures, it probably has a structural function [Compton et al., 1992; Yang et al., 1992]. NuMA is a component of a subset of nuclear matrix filaments [Zeng et al., 1994b] and has been shown to be associated with splicing complexes [Zeng et al., 1994a; reviewed by Cleveland, 1995]. Like hnRNP-U, NuMA does not migrate well in focusing gels, so we detected it by immunoblotting after one-dimensional SDS–PAGE (Fig. 7). We found that all NuMA protein is associated with the nuclear matrix preparation. About 50% of this protein remains after dissociation of the internal nuclear matrix and, therefore, may be associated to the nuclear shell. This observation is in agreement with in situ immunolabeling, which shows that NuMA is not only present throughout the nucleoplasm, but is also associated with the periphery of the nucleus [see Figure 2A in Compton et al., 1992].

DISCUSSION

The internal nuclear matrix is a fibrogranular structure that is present throughout the nucleoplasm and can be visualized after removal of most chromatin and loosely bound nuclear components. The molecular structure of this fibrogranular network inside the nucleus is still an enigma [Cook, 1988; Jack and Eggert, 1992; Stuurman et al., 1992a]. Many components involved in a variety of nuclear processes, like

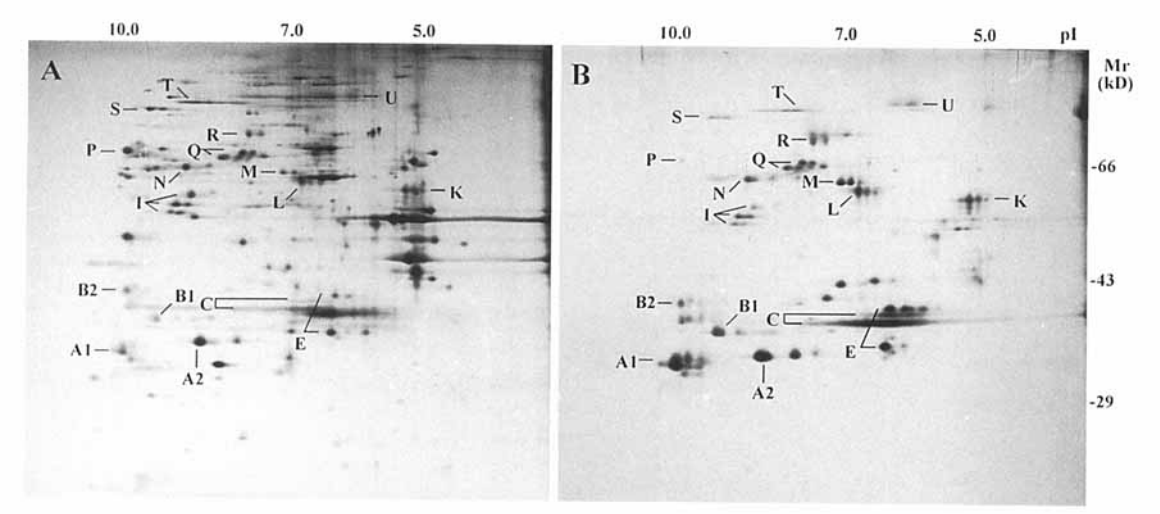

Fig. 6. Comparison of nuclear matrix proteins with hnRNP proteins. Proteins of nuclear matrices (**A**) and proteins of immunopurified hnRNP complexes (**B**) were separated simultaneously by NEPHGE/SDS–PAGE and detected by silver staining. hnRNP proteins, present in both preparations, are indicated according to the nomenclature of Piñol-Roma et al. [1988].

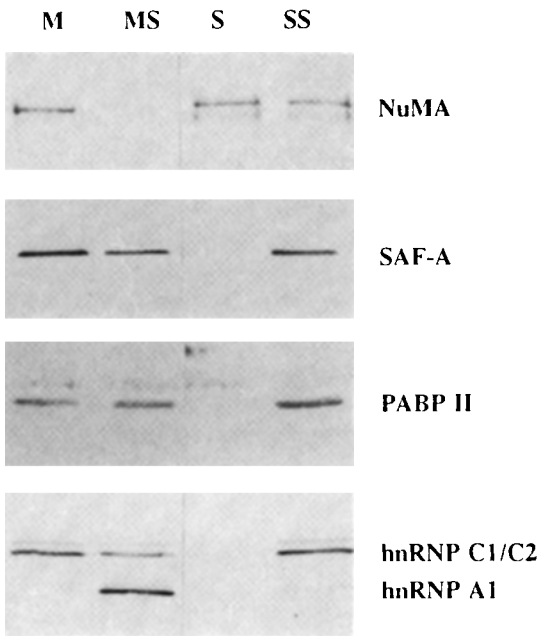

Fig. 7. Retention of several internal nuclear matrix proteins by nuclear matrices and shells. Proteins of nuclear subfractions were separated by SDS–PAGE and transferred to nitrocellulose. Blots were immunostained with antibodies against NuMA, SAF-A/hnRNP-U, PAB II, hnRNP-C1/C2, and hnRNP-A1. Each lane contains protein from 10^6 nuclear equivalents. The lanes contain nuclear matrices (M), proteins extracted by DNase digestion and 0.25 M ammonium sulfate (MS), nuclear shells (S), and proteins extracted by RNase digestion, 40 mM DTT, and 2 M NaCl (SS).

replication, transcription, RNA processing and RNA transport, have been shown to bind to the nuclear matrix [reviewed by Berezney, 1991; Getzenberg, 1994; van Driel et al., 1991, 1995; Verheijen et al., 1988]. If the basic structure of the internal nuclear matrix is made up of filamentous proteins, analogous to the cytoskeleton and the nuclear lamina, structural proteins of the internal matrix are expected to be relatively abundant, similar to cytoskeletal proteins and those of the nuclear lamina. However, no systematic analysis has been carried out yet to designate the quantitatively major matrix proteins. To identify proteins that are candidates for structural components of the internal matrix, we set out to analyze the protein composition of the internal nuclear matrix quantitatively.

Here we describe a method that allows us to identify the internal nuclear matrix proteins in HeLa S3 cells. We made use of the fact that it is possible to dissociate the internal nuclear matrix selectively, leaving the nuclear lamina (shell) intact [Belgrader et al., 1991; Kaufmann and Shaper, 1984; de Graaf et al., 1992; Stuurman et al., 1990]. The protein composition of nuclear matrices and of nuclear shells were compared by quantitative two-dimensional gel analysis. Proteins present in intact nuclear matrix preparations and essentially absent (i.e., less than 10% remains) in nuclear shells are probably uniquely present in the internal matrix.

Using this approach, we identified 56 protein spots in IEF gels and 64 in NEPHGE gels that

relate to proteins exclusively present in preparations that contain the internal nuclear matrix. Most of these proteins are of relatively low abundance. Table I lists the 21 most abundant proteins of the internal matrix, together representing about 75% of the total amount of internal matrix protein. We show that of these 21 proteins 16 are hnRNP proteins and one is B23 (numatrin, nucleophosmin). Four proteins are yet unidentified (Table I).

Most hnRNP proteins were not identified earlier as nuclear matrix proteins. Fey et al. [1986] found that many hnRNP proteins were components of the nuclear matrix, but did not identify them individually. He et al. [1991] showed that antibodies to hnRNP core proteins (the A, B, and C proteins) labeled the granular structures of the internal nuclear matrix, rather than the filaments. Also hnRNP-U (also called SAF-A) has been shown earlier to be a constituent of the nuclear matrix [Romig et al., 1992]. The hnRNP proteins are major components of the nucleus. For example, there are about 1×10^6 copies of hnRNP-U per nucleus [Romig et al., 1992]. Clearly, hnRNP proteins are the major internal nuclear matrix proteins.

hnRNP proteins are not retained completely in nuclear matrix preparations and different hnRNP proteins are retained to different extents. For instance, most of hnRNP-A1 is solubilized upon treatment of nuclei with DNase I and high salt, whereas more than 50% of hnRNP-C and hnRNP-U are bound to the nuclear matrix. Apparently, there are different pools of hnRNP protein. The function of hnRNP proteins is not completely clear. They are believed to play an important role in the packaging, processing and transport of mRNA [reviewed by Dreyfuss et al., 1993]. For example, hnRNP I has been shown to be the polypyrimidine tract binding protein (PTB) [Ghetti et al., 1992]. Since some hnRNP proteins are able to form filamentous structures under certain conditions [Lothstein et al., 1985; Romig et al., 1992] and some are able to bind DNA, they may also have a role in determining chromatin structure and in the control of gene expression. For instance, hnRNP-U is able to bind scaffold/matrix-associated regions (S/MARs), which are AT-rich sequences by which chromatin loops are attached to the nuclear matrix [Romig et al., 1992; von Kries et al., 1994]. Other hnRNP proteins have been shown to be able to interact with telomeres [Ishikawa et al., 1993]. Finally, hnRNP-K is shown to be a DNA-binding protein and transcriptional activator [Tomonaga and Levens, 1995]. Because the internal nuclear matrix consists mainly of hnRNP proteins, it probably is involved in RNA metabolism.

B23 is a very abundant protein in the internal nuclear matrix (Table I). B23 is a nucleolar phosphoprotein, also called numatrin or nucleophosmin. B23 is enriched in proliferating cells [Feuerstein and Mond, 1987]. It has been reported that there are approximately 6×10^6 molecules of B23 per HeLa cell [Yung and Chan, 1987]. Like hnRNP proteins, it is involved in RNA processing and transport. Immunoelectron microscopy studies have shown that B23 is localized in the granular regions of nucleoli where ribosomes are assembled [Spector et al., 1984]. The ribonuclease activity of B23 suggests that it plays a role in the processing of preribosomal RNA [Herrera et al., 1995]. Also, it may be involved in ribosome transport and assembly. Borer et al. [1989] found that it shuttles between nucleus and cytoplasm. Recently, the protein has been shown to be present in granules throughout the nucleoplasm in addition to its nucleolar localization [J.M. Bridger, M.E. Kerr, and C.J. Hutchison, personal communication]. These observations indicate that B23, besides in the nucleolar remnants, may also be present in the fibrogranular structures of the nuclear matrix.

The protein composition of the nuclear matrix has been studied by several investigators, using different cell types and matrix isolation methods [e.g., Fey et al., 1986; Kallajoki and Osborn, 1994; Kaufmann and Shaper, 1984; Verheijen et al., 1986]. Stuurman et al. [1990] identified a set of matrix proteins that are found in several cell types and species and called this the minimal matrix. However, a systematic attempt to identify quantitatively a set of proteins that form specifically the fibrogranular internal nuclear matrix has not been made before. Some investigators also find that hnRNPs and B23 are components of the nuclear matrix, like Nakayasu and Berezney [1991], who identified 12 major nuclear matrix proteins, the matrins. Because of differences in matrix isolation and electrophoretic techniques, it is difficult to compare our results with that of others.

We have formulated an objective criterium for the identification of proteins that may be constituents of the internal, fibrogranular nuclear matrix. Although the criterium is simple and

straightforward, it may result in some false-negatives and false-positives. There are several reasons for this: (1) proteins may not be detected adequately by silver staining; (2) proteins may not show up on two-dimensional gels because they have extreme physical properties (e.g., extremely basic, acidic, large or small proteins); (3) proteins may be missed on two-dimensional gels because they are poorly soluble or move too slowly under conditions that we run the first electrophoretic dimension; (4) some proteins that are part of the internal nuclear matrix may remain associated to the nuclear shells after selective dissociation of the internal matrix; and (5) proteins from other structures than the internal nuclear matrix, e.g., the cytoskeleton and the nuclear lamina, may become soluble under conditions supposed to dissociate the internal matrix selectively. An example of false negatives is NuMA. This protein could not be detected on two-dimensional gels, most probably because it focuses poorly. The fractionation behaviour of this protein could be analyzed by immunoblotting after one-dimensional SDS–PAGE. Results show that NuMA partitions about equally over the lamina-pore complex and the internal nuclear matrix. This observation is in agreement with an observation of [Compton et al., 1992], showing by immunofluorescence that NuMA is present in the nucleoplasm, but also seems to be associated to the nuclear lamina. Also, we find that about 25% of the lamin A and lamin C proteins is lost under conditions that the internal matrix is dissociated, whereas lamin B1 was fully retained in nuclear shell preparations. This is consistent with observations that lamin A/C antigen is present in the nucleoplasm [Bridger et al., 1993; Hozák et al., 1995]. Alternatively, some lamin A and lamin C protein may simply dissociate from the lamina-pore structure. Also a small amount of actin and keratin 18 (less than 10%) appeared to dissociate under the reducing conditions used to dissociate the internal nuclear matrix. This may indicate that these proteins occur in the internal matrix. Actin is present in the nucleus and may therefore be a component of the nuclear matrix (reviewed by [Mattern et al., 1996; Verheijen et al., 1988]. However, it is equally possible that these proteins were dissociated from the cytoskeleton that is present in the nuclear matrix preparations and, therefore, are not part of the nuclear matrix. Evidently, the precise localization of putative internal matrix proteins should be confirmed by in situ immunolabeling studies. Despite these potential pitfalls, it is very likely that most, if not all, of the set of most abundant putative internal matrix proteins are really major components of this structure. B23 and hnRNP proteins are genuine major nuclear proteins. Only PABP I and any of the four yet unidentified proteins (Table I) may not be part of the nuclear matrix. Further studies are under way to answer these questions.

What can we conclude from these results about the structure and function of the internal nuclear matrix? We identified 17 of the 21 most abundant internal matrix proteins, B23 and 16 hnRNP proteins. Importantly, these proteins are involved in packaging, processing and transport of RNA [Dreyfuss et al., 1993]. Moreover, about 70% of the nuclear RNA is still present in nuclear matrix preparations, if RNase activity is suppressed during isolation [He et al., 1990; van Eekelen and van Venrooij, 1981]. Also, transcription and RNA processing activity is preserved in the nuclear matrix [Jackson and Cook, 1985; Razin and Yarovaya, 1985; Zeitlin et al., 1989]. Together, these observations support the notion that the internal nuclear matrix is a structure that is strongly committed to RNA metabolism and transport.

Several observations indicate the presence of intermediate filament-like structures in the internal nuclear matrix. Immuno-electron microscopy showed that NuMA and lamin A/C are locally present in intranuclear fibrogranular structures [Hozák et al., 1995; Zeng et al., 1994b]. Although it cannot be ruled out that NuMA and/or other yet unknown matrix proteins constitute a proteinaceous network in the nucleoplasm, it may be that the fibrogranular structure of the nuclear matrix is an aggregate of RNA-protein complexes. The fibrogranular nature of these aggregates either may reflect an intrinsic property of the constituting RNPs or is imposed by the shape of the interchromatin space in which RNA synthesis, processing and transport seem to take place [Cremer et al., 1993; Mattern et al., 1996]. If the fibrogranular structure of the nuclear matrix is merely an aggregate of RNA-protein complexes, the internal nuclear matrix is expected to be absent in nuclei of cells that are not transcriptionally active. Indeed, several agents that inhibit or stimulate transcription induce extensive structural rearrangements in nuclei and nuclear matrices. It is also found that in dormant nuclei, like

those of avian erythrocytes, the internal nuclear matrix elements are greatly reduced [reviewed by Brasch, 1990]. So, the internal nuclear matrix may not be a structure that organizes the cell nucleus in an active manner. Rather, the internal matrix seems to be shaped by RNA synthesis, processing, and transport.

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REFERENCES

Amankwah KS, Deboni U (1994): Ultrastructural localization of filamentous actin within neuronal interphase nuclei in situ. Exp Cell Res 210:315–325.

Belgrader P, Siegel AJ, Berezney R (1991): A comprehensive study on the isolation and characterization of the HeLa S3 nuclear matrix. J Cell Sci 98:281–291.

Berezney R (1991): The nuclear matrix: a heuristic model for investigating genomic organization and function in the cell nucleus. J Cell Biochem 47:109–123.

Berezney R, Coffey DS (1974): Identification of a nuclear protein matrix. Biochem Biophys Res Commun 60:1410–1417.

Blencowe BJ, Nickerson JA, Issner R, Penman S, Sharp PA (1994): Association of nuclear matrix antigens with exon-containing splicing complexes. J Cell Biol 127:593–607.

Borer RA, Lehner CF, Eppenberger HM, Nigg EA (1989): Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell 56:379–390.

Brasch K (1990): Drug and metabolite-induced perturbations in nuclear structure and function: a review. Biochem Cell Biol 68:408–426.

Bridger JM, Kill IR, O'Farrell M, Hutchison CJ (1993): Internal lamin structures within G1 nuclei of human dermal fibroblasts. J Cell Sci 104:297–306.

Burke B, Tooze J, Warren G (1983): A monoclonal antibody which recognises each of the nuclear lamin polypeptides in mammalian cells. EMBO J 2:361–367.

Capco DG, Wan KM, Penman S (1982): The nuclear matrix: three-dimensional architecture and protein composition. Cell 29:847–858.

Celis JE, Rasmussen HH, Olsen E, Madsen P, Leffers H, Honore B, Dejgaard K, Gromov P, Hoffmann HJ, Nielsen M, Vassilev A, Vintermyr O, Hao J, Celis A, Basse B, Lauridsen JB, Ratz GP, Andersen AH, Walbum E, Kjaergaard I, Puype M, Vandamme J, Vandekerckhove J (1993): The human keratinocyte 2-dimensional gel protein database: update 1993. Electrophoresis 14:1091–1198.

Celis JE, Rasmussen HH, Olsen E, Madsen P, Leffers H, Honore B, Dejgaard K, Gromov P, Vorum H, Vassilev A, Baskin Y, Liu XD, Celis A, Basse B, Lauridsen JB, Ratz GP, Andersen AH, Walbum E, Kjaergaard I, Andersen I, Puype M, Vandamme J, Vandekerckhove J (1994): The human keratinocyte two-dimensional protein database (update 1994): Towards an integrated approach to the study of cell proliferation, differentiation and skin diseases. Electrophoresis 15:1349–1458.

Choi YD, Dreyfuss G (1984): Monoclonal antibody characterization of the C proteins of heterogeneous nuclear ribonucleoprotein complexes in vertebrate cells. J Cell Biol 99: 1997–2004.

Cleveland DW (1995): NuMA: A protein involved in nuclear structure, spindle assembly, and nuclear re-formation. Trends Cell Biol 5:60–64.

Cleveland DW, Fischer SG, Kirscher MW, Laemmli UK (1977): Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J Biol Chem 252:1102–1106.

Compton DA, Szilak I, Cleveland DW (1992): Primary structure of NuMA, an intranuclear protein that defines a novel pathway for segregation of proteins at mitosis. J Cell Biol 116:1395–1408.

Cook PR (1988): The nucleoskeleton: artefact, passive framework or active site? J Cell Sci 90:1–6.

Cook PR (1991): The nucleoskeleton and the topology of replication. Cell 66:627–635.

Cremer T, Kurz A, Zirbel R, Dietzel S, Rinke B, Schrock E, Speicher MR, Mathieu U, Jauch A, Emmerich P, Scherthan H, Ried T, Cremer C, Lichter P (1993): Role of chromosome territories in the functional compartmentalization of the cell nucleus. Cold Spring Harbor Symp Quant Biol 58:777–792.

De Graaf A, Meijne AML, van Renswoude AJBM, Humbel BM, van Bergen en Henegouwen PMP, de Jong L, van Driel R, Verkleij AJ (1992): Heat shock induced redistribution of a 160 kDa nuclear matrix protein. Exp Cell Res 202:243–251.

De Jong L, van Driel R, Stuurman N, Miejne AML, van Renswoude J (1990): Principles of nuclear organization. Cell Biol Int Rep 14:1051–1074.

Dessev GN (1990): The nuclear lamina—An intermediate filament protein structure of the cell nucleus. In Goldman RD, Steinert PM (eds): "Cellular and Molecular Biology of Intermediate Filaments." New York: Plenum Press, pp 129–145.

Dreyfuss G, Matunis MJ, Piñol-Roma S, Burd CG (1993): hnRNP Proteins and the biogenesis of mRNA. Annu Rev Biochem 62:289–321.

Fackelmayer FO, Dahm K, Renz A, Ramsperger U, Richter A (1994): Nucleic-acid-binding properties of hnRNP-U/SAF-A, a nuclear-matrix protein which binds DNA and RNA in vivo and in vitro. Eur J Biochem 221:749–757.

Feuerstein N, Mond JJ (1987): "Numatrin": A nuclear matrix protein associated with induction of proliferation in B lymphocytes. J Biol Chem 262:11389–11397.

Fey EG, Krochmalinik G, Penman S (1986): The nonchromatin substructures of the nucleus: The ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionating and resinless section electron microscopy. J Cell Biol 102:1654–1665.

Fey EG, Bangs P, Sparks C, Odgren P (1991): The nuclear matrix: defining structural and functional roles. CRC Crit Rev Eukaryote Gene Expres 1:127–143.

Fields AP, Kaufmann SH, Shaper JH (1986): Analysis of the internal nuclear matrix. Exp Cell Res 164:139–153.

Georgatos SD, Meier J, Simos G (1994): Lamins and lamin-associated proteins. Curr Opin Cell Biol 6:347–353.

Gerace L, Burke B (1988): Functional organization of the nuclear envelope. Annu Rev Cell Biol 4:335–374.

Gersten DM, Rodriguez LV, George DG, Johnston DA, Zapolski EJ (1991): On the relationship of amino acid composition to silver staining of proteins in electrophoresis gels. II. Peptide sequence analysis. Electrophoresis 12:409–414.

Getzenberg RH (1994): Nuclear matrix and the regulation of gene expression: Tissue specificity. J Cell Biochem 55: 22–31.

Getzenberg RH, Pienta KJ, Ward WS, Coffey DS (1991): Nuclear structure and the three-dimensional organization of DNA. J Cell Biochem 47:289–299.

Ghetti A, Piñol-Roma S, Michael WM, Morandi C, Dreyfuss G (1992): hnRNP I, the polypyrimidine tract-binding protein: Distinct nuclear localization and association with hnRNAs. Nucleic Acids Res 20:3671–3678.

Görlach M, Burd CG, Dreyfuss G (1994): The mRNA poly(A)-binding protein: localization, abundance, and RNA-binding specificity. Exp Cell Res 211:400–407.

Harlow E, Lane D (1988): "Antibodies: A Laboratory Manual." Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

He D, Nickerson JA, Penman S (1990): Core filaments of the nuclear matrix. J Cell Biol 110:569–580.

He DC, Martin T, Penman S (1991): Localization of heterogeneous nuclear ribonucleoprotein in the interphase nuclear matrix core filaments and on perichromosomal filaments at mitosis. Proc Natl Acad Sci USA 88:7469–7473.

Herrera JE, Savkur R, Olson MOJ (1995): The ribonuclease activity of nucleolar protein B23. Nucleic Acids Res 23: 3974–3979.

Heukeshoven J, Dernick R (1986): In Radola BJ (ed): "Elektrophorese Forum '86." München: Technische Universität, pp 22–27.

Hozák P, Sasseville AMJ, Raymond Y, Cook PR (1995): Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. J Cell Sci 108:635–644.

Humbel B, Müller M (1986): Freeze-substitution and low temperature embedding. In Müller M, Bedu RP, Boyle A, Wolosewich JJ (eds): "Science of Biological Specimen Preparation." O'Hare: SEM Inc AMF, pp 175–183.

Hutchison CJ, Bridger JM, Cox LS, Kill IR (1994): Weaving a pattern from disparate threads: Lamin function in nuclear assembly and DNA replication. J Cell Sci 107: 3259–3269.

Ishikawa F, Matunis MJ, Dreyfuss G, Cech TR (1993): Nuclear proteins that bind the pre-messenger RNA 3′ splice site sequence r(UUAG/G) and the human telomeric DNA sequence d(TTAGGG)n. Mol Cell Biol 13:4301–4310.

Jack RS, Eggert H (1992): The elusive nuclear matrix. Eur J Biochem 209:503–509.

Jackson DA, Cook PR (1985): Transcription occurs at a nucleoskeleton. EMBO J 4:919–925.

Jackson DA, Cook PR (1986): Replication occurs at a nucleoskeleton. EMBO J 5:1403–1410.

Jackson DA, Cook PR (1988): Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat. EMBO J 7:3667–3677.

Kallajoki M, Osborn M (1994): Gel electrophoretic analysis of nuclear matrix fractions isolated from different human cell lines. Electrophoresis 15:520–528.

Kallajoki M, Weber K, Osborn M (1991): A 210 kDa nuclear matrix protein is a functional part of the mitotic spindle—a microinjection study using SPN monoclonal antibodies. EMBO J 10:3351–3362.

Kaufmann HS, Shaper JH (1984): A subset of non-histone nuclear proteins reversibly stabilized by the sulfhydryl cross-linking reagent tetrathionate. Exp Cell Res 155:477–495.

Kaufmann SH, Coffey DS, Shaper JH (1981): Considerations in the isolation of rat liver nuclear matrix, nuclear envelope, and pore complex lamina. Exp Cell Res 132:105–123.

Krause S, Fakan S, Weis K, Wahle E (1994): Immunodetection of poly(A) binding protein II in the cell nucleus. Exp Cell Res 214:75–82.

Laemmli UK (1970): Cleavage of structural proteins during assembly of the head bacteriophage T4. Nature 227:680–685.

Lothstein L, Arenstorf HP, Chung SY, Walker BW, Wooley JC, Le Stourgeon WM (1985): General organization of proteins in HeLa 40S nuclear ribonucleoproteins particles. J Cell Biol 100:1570–1581.

Ludérus MEE, de Graaf A, Mattia E, den Blaauwen JL, Grande MA, de Jong L, van Driel R (1992): Binding of matrix attachment regions to lamin-B1. Cell 70:949–959.

Lydersen BK, Pettijohn DE (1980): Human-specific nuclear protein that associates with the polar region of the mitotic apparatus: distribution in a human/hamster hybrid cell. Cell 22:489–499.

Mattern KA, de Jong L, van Driel R (1996): Composition and structure of the internal nuclear matrix. In Bird RC, Stein G, Lian J, Stein J (eds): "Nuclear Structure Gene Expression." San Diego: Academic Press (in press).

Meissner DH, Schwarz H (1990): Improved cryofixation and freeze-substitution of embryonic quail retina: A TEM study on ultrastructural preservation. J Electron Microsc Technol 14:348–356.

Mirkovitch J, Mirault M-E, Laemmli UK (1984): Organization of the higher-order chromatin loop: Specific DNA attachment sites on nuclear scaffold. Cell 39:223–232.

Nakayasu H, Berezney R (1989): Mapping replication sites in the eucaryotic nucleus. J Cell Biol 108:1–11.

Nakayasu H, Berezney R (1991): Nuclear matrins—Identification of the major nuclear matrix proteins. Proc Nat Acad Sci USA 88:10312–10316.

Neri LM, Riederer BM, Marugg RA, Capitani S, Martelli AM (1995): The effect of sodium tetrathionate stabilization on the distribution of three nuclear matrix proteins in human K562 erythroleukemia cells. Histochem Cell Biol 104:29–36.

Nigg EA (1989): The nuclear envelope. Curr Opin Cell Biol 1:435–440.

Penman S (1995): Rethinking cell structure. Proc Natl Acad Sci USA 92:5251–5257.

Piñol-Roma S, Choi YD, Matunis MJ, Dreyfuss G (1988): Immunopurification of heterogeneous nuclear ribonucleoprotein particles reveals an assortment of RNA-binding proteins. Genes Dev 2:215–227.

Piñol-Roma S, Swanson MS, Gall JG, Dreyfuss G (1989): A novel heterogeneous nuclear RNP protein with a unique distribution on nascent transcripts. J Cell Biol 109:2575–2587.

Piñol-Roma S, Choi YD, Dreyfuss G (1990): Immunological methods for purification and characterization of heterogeneous nuclear ribonucleoprotein particles. Methods Enzymol 181:317–325.

Rabilloud T (1992): A comparison between low background silver diammine and silver nitrate protein stains. Electrophoresis 13:429–439.

Ramaekers F, Huysmans A, Schaart G, Moesker O, Vooijs P (1987): Tissue distribution of keratin 17 as monitored by a monoclonal antibody. Exp Cell Res 170:235–249.

Razin SV, Yarovaya OV (1985): Initiated complexes of RNA polymerase II are concentrated in the nuclear skeleton associated DNA. Exp Cell Res 158:273–275.

Romig H, Fackelmayer FO, Renz A, Ramsperger U, Richter A (1992): Characterization of SAF-A, a novel nuclear DNA binding protein from HeLa cells with high affinity for nuclear matrix/scaffold attachment DNA elements. EMBO J 11:3431–3440.

Spector DL, Ochs RL, Busch H (1984): Silver staining, immunofluorescence, and immunoelectron microscopic localization of nucleolar phosphoproteins B23 and C23. Chromosoma 90:139–148.

Stein GS, Lian JB, Dworetzky SI, Owen TA, Bortell R, Bidwell JP, van Wijnen AJ (1991): Regulation of transcription-factor activity during growth and differentiation involvement of the nuclear matrix in concentration and localization of promoter binding proteins. J Cell Biochem 47:300–305.

Stein GS, van Wijnen AJ, Stein JL, Lian JB, Bidwell JP, Montecino M (1994): Nuclear architecture supports integration of physiological regulatory signals for transcription of cell growth and tissue-specific genes during osteoblast differentiation. J Cell Biochem 55:4–15.

Stuurman N, Meijne AML, van der Pol AJ, de Jong L, van Driel R, van Renswoude J (1990): The nuclear matrix from cells of different origin—evidence for a common set of matrix proteins. J Biol Chem 265:5460–5465.

Stuurman N, de Jong L, van Driel R (1992a): Nuclear frameworks—Concepts and operational definitions. Cell Biol Int Rep 16:837–852.

Stuurman N, Floore A, Colen A, de Jong L, van Driel R (1992b): Stabilization of the nuclear matrix by disulfide bridges—Identification of matrix polypeptides that form disulfides. Exp Cell Res 200:285–294.

Swanson MS, Dreyfuss G (1988): Classification and purification of heterogeneous nuclear ribonucleoprotein particles by RNA-binding specificities. Mol Cell Biol 8:2237–2241.

Tomonaga T, Levens D (1995): Heterogeneous nuclear ribonucleoprotein K is a DNA-binding transactivator. J Biol Chem 270:4875–4881.

Towbin H, Staehelin T, Gordon J (1979): Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedures and some applications. Proc Natl Acad Sci USA 76:4350–4354.

Van Driel R, Humbel B, de Jong L (1991): The nucleus—A black box being opened. J Cell Biochem 47:311–316.

Van Driel R, Wansink DG, van Steensel B, Grande MA, Schul W, de Jong L (1995): Nuclear domains and the nuclear matrix. Int Rev Cyt: 151–189.

Van Eekelen CAG, van Venrooij WJ (1981): hnRNA and its attachment to the nuclear protein matrix. J Cell Biol 88:554–563.

Van Steensel B, Jenster G, Damm K, Brinkmann AO, van Driel R (1995): Domains of the human androgen receptor and glucocorticoid receptor involved in binding to the nuclear matrix. J Cell Biochem 57:465–478.

Venable JH, Coggeshall R (1965): A simplified lead citrate stain for use in electron microscopy. J Cell Biol 25:407–408.

Verheijen R, Kuijpers H, Vooijs P, van Venrooij W, Ramaekers F (1986): Protein composition of nuclear matrix preparations from HeLa cells: an immunochemical approach. J Cell Sci 80:103–122.

Verheijen R, van Venrooij W, Ramaekers F (1988): The nuclear matrix: structure and composition. J Cell Sci 90:11–36.

Von Kries JP, Buck F, Stratling WH (1994): Chicken MAR binding protein P120 is identical to human heterogeneous nuclear ribonucleoprotein (hnrnp) U. Nucleic Acids Res 22:1215–1220.

Wahle E, Lustig A, Jenö P, Maurer P (1993): Mammalian poly(A)-binding protein II—Physical properties and binding to polynucleotides. J Biol Chem 268:2937–2945.

Wang D, Umekawa H, Olson MOJ (1993): Expression and subcellular locations of two forms of nucleolar protein B23 in rat tissues and cells. Cell Mol Biol Res 39:33–42.

Wansink DG, Sibon OCM, Cremers FFM, van Driel R, de Jong L (1996): Ultrastructural localization of active genes in nuclei of A431 cells. J Cell Biochem (in press).

Yang CH, Lambie EJ, Snyder M (1992): NuMA—An unusually long coiled-coil related protein in the mammalian nucleus. J Cell Biol 116:1303–1317.

Yung BYM, Chan PK (1987): Identification and characterization of a hexameric form of nucleolar phosphoprotein B23. Biochim Biophys Acta 925:74–82.

Zeitlin S, Wilson RC, Efstratiadis A (1989): Autonomous splicing and complementation of in vivo-assembled spliceosomes. J Cell Biol 108:765–777.

Zeng C, He D, Berget SM, Brinkley BR (1994a): Nuclear-mitotic apparatus protein: a structural protein interface between the nucleoskeleton and RNA splicing. Proc Natl Acad Sci USA 91:1505–1509.

Zeng CQ, He DC, Brinkley BR (1994b): Localization of NuMA protein isoforms in the nuclear matrix of mammalian cells. Cell Motility Cytoskel 29:167–176.