The Nuclear Matrix from Cells of Different Origin

EVIDENCE FOR A COMMON SET OF MATRIX PROTEINS*

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We compared the protein composition of the nuclear matrix isolated from several murine embryonal carcinoma cells and mature tissues by two-dimensional gel electrophoresis. Two nuclear matrix fractions were investigated: the “peripheral” nuclear matrix (matrix proteins that remain insoluble after reduction), and the “internal” nuclear matrix (matrix proteins released by reduction). The two subfractions have completely different protein compositions. Although numerous differences in nuclear matrix protein composition among different cell types were observed, a limited set of polypeptides common to all mouse cell types was identified. A majority of these common proteins was also present in cells from other mammalian species (i.e. rat and human). For this set of proteins, we coin the term “minimal matrix.”

As expected, lamin B, known to be expressed throughout differentiation, is part of the common set of peripheral nuclear matrix proteins. Lamins A and C are not because these proteins were absent from undifferentiated embryonal carcinoma cells. Since these common nuclear matrix proteins occur in all mammalian nuclear matrices analyzed so far, it is likely that they have a basic role in nuclear organization and function.

Recently, several authors have expanded on the idea that a proteinaceous network in the nucleus (the nuclear matrix) is involved in control and coordination of gene expression during differentiation (Blobel, 1985; Razin, 1987; Bodnar, 1988). It is thought that loops of chromatin are persistently as well as transiently attached to this network (Piñata and Coffey, 1984; Mirkovitch et al., 1984; Gassar and Laemmli (1987); Earnshaw, 1988). It has been proposed that each chromatin loop constitutes an independent regulatory unit containing several genes. Processes such as DNA replication (Jackson et al., 1984; Dijkwel et al., 1986; Razin et al., 1986; Tubo and Martelli, 1987; Nakayasu and Berzemez, 1988), transcription (Jackson and Cook, 1985; Razin et al., 1985; Bottyan and Olsson, 1986), RNA processing and transport (Schröder et al., 1987a, 1987b; Zeitlin et al., 1987), and the regulation of DNA superhelicity (Herios et al., 1986) have been shown to be associated with this nuclear matrix. These observations support the hypothesis that the nuclear matrix is instrumental in regulation and coordination of gene expression.

Little is known about the nature of the proteins that constitute the nuclear matrix. It seems likely that the nuclear matrix has a similar basal structure in almost all mammalian cells. Therefore, we expect that the elements essential to this structure will tend to be conserved during evolution and remain present throughout differentiation. In addition, numerous differentiation state- and species-specific components will be present in each nuclear matrix.

Recently, we and others (Fey and Penman, 1987, 1988; Stuurman et al., 1989) have shown that the protein composition of the nuclear matrix is differentiation state dependent. Here, we present a detailed analysis of the matrix composition determined by two-dimensional gel electrophoresis (O’Farrell, 1975; O’Farrell et al., 1977). Nuclear matrices were prepared by extraction of nuclease-treated purified nuclei with buffers of high ionic strength. Two matrix subfractions were isolated, one enriched in nuclear envelope polypeptides (peripheral matrix), the other containing mainly internal matrix proteins (internal matrix) (Kauffmann and Shaper, 1984). We have looked for nuclear matrix polypeptides that are present in a variety of murine cell types and tissues and in cells that originate from other mammals (rat and human). The results show that a limited set of proteins is present in the nuclear matrix of all cell types studied, irrespective of species and differentiation state. We propose that these proteins are essential in constituting the nuclear matrix and coin the term minimal matrix for them.

MATERIALS AND METHODS

Cell Culture

P19EC (Mc Burney et al., 1982), P19MES-1 (Mummery et al., 1986), P19EPI-7, and P19END-2 (Mummery et al., 1988) cells were grown in monolayers in 0.1% (w/v) gelatin-coated tissue culture flasks in 45% (v/v) Dulbecco’s minimum essential medium, 45% (v/v) Ham’s F-12, and 10% (v/v) fetal bovine serum (GIBCO) buffered with bicarbonate in a 5% CO₂ atmosphere at 37 °C. F9 (a gift from Dr. B. Terrana, Sclavo Research Center, Siena, Italy), P9AC c19 (Howe and Solter, 1981), Dif-5 (Nagarajan et al., 1983), PYS-2 (Lehman et al., 1974), and PC13 (Bernstine et al., 1973) cells were cultured in 90% (v/v) Dulbecco’s minimum essential medium, 10% (v/v) fetal calf serum in noncoated tissue culture flasks. All these cell lines originate from mice. Cells were subcultured using 0.05% (w/v) trypsin, 0.02% (w/v) EDTA (Flow Labs, Irvine, Ayrshire, United Kingdom). Human K562 erythroleukemia cells were grown in suspension in 90% (v/v) RPMI 1640, 10% newborn calf serum (GIBCO). P19EC cells, growing in monolayer culture, were induced to differentiate by addition to the culture medium of all-trans-retinoic acid (Sigma) from a stock solution (10⁻⁸ M in dimethyl sulfoxide) to a final concentration of 10⁻⁶ M.

Isolation of Nuclei

Nuclei of tissue culture cells were isolated as described (Rao et al., 1986). Briefly, phosphate-buffered saline (150 mM NaCl, 6.7 mM Na₂HPO₄, 1.5 mM KH₂PO₄),...
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Nuclear Matrix Proteins

It is important to avoid confusion about the nature and the nomenclature of the nuclear matrix fractions used in this study. Basically two kinds of fractions were employed. One type of fraction resulted from applying a classical protocol for isolating nuclear matrices, including an extraction with a reducing agent (Kaufmann et al., 1983). Since the work of Kaufmann et al. (1983) has shown that the residues of nuclei in this type of fraction appear as rather empty ghosts, this fraction will be referred to as peripheral nuclear matrix throughout this paper. The other type of fraction used consists of the material released upon treatment of the matrices with reducing agents and is called internal nuclear matrix in agreement with the designation given by Kaufmann and Shaper (1984).

Isolation of peripheral nuclear matrices

Peripheral nuclear matrices were isolated from purified nuclei by the aforementioned method of Kaufmann et al. (1983) with minor modifications. Sucrose-cushion-purified nuclei were resuspended in 0.25 mg/ml DNase I (from bovine pancreas, 2000 Kunitz units/mg; Sigma) and 0.25 mg/ml RNase A (type I-AS from bovine pancreas, 50–100 Kunitz units/mg; Sigma) in STM, supplemented with 1 mM PMSF and aprotinin at 100 kallikrein-inactivating units/ml, to a density of 3 × 10⁹ nuclei/ml in LS (low salt) buffer (10 mM Tris/HC1 (pH 7.4 at 4°C), 5 mM MgSO₄, 1 mM PMSF, supplemented with 0.2 mM PMSF, and the nuclei were layered on sucrose cushions and centrifuged as described above.

Isolation of Nuclei

Isolation of nuclei from tissues was essentially according to Blobel and Potter (1966). Freshly excised mouse uterine tissues (or, alternatively, tissues that had been stored at −70°C) were minced in STM (250 mM sucrose, 50 mM Tris/HC1 (pH 7.4 at 4°C), 5 mM MgSO₄, 1 mM PMSF) supplemented with 0.2 mM PMSF, washed three times with the same buffer by decantation, and subsequently homogenized at 4°C using a motor-driven Potter-Elvehjem homogenizer equipped with a Teflon pestle (10 strokes at 500 rpm followed by 5 strokes at 1,000 rpm). After centrifugation at 1,000 × g, postnuclear supernatant was separated from the pellet. The pellet was washed three times with STM supplemented with 0.2 mM PMSF, and the nuclei were layered on sucrose cushions and centrifuged as described above.

Isolation of the Nuclear Matrix

Protein Composition

Protein compositions were determined using the microassay described by Peterson (1977). Twenty or fifty micrograms of nuclear matrix protein was suspended in lysis buffer (as described by O’Farrell, 1975). After 30 min, insoluble material was removed by centrifugation at 10,000 × g (pellet and supernatant had a similar protein composition as judged by SDS-PAGE, indicating that the composition of the supernatant is similar to the composition of the total matrix preparation) and layered on a NEPHGE tube gel as described by Bravo (1984). After electrophoresis (4.5 h at 400 V), the tube gels were placed on top of an 8% SDS-polyacrylamide gel, and gel electrophoresis was carried out according to Laemmli (1970). The gels were stained with silver, employing the method of Morrissey (1981).

RESULTS

Protein Composition of the Peripheral Nuclear Matrix from Various Mouse Embryonal Carcinoma Cell Lines—Nuclei of tissue culture cells were isolated by hypotonic lysis and centrifugation through a sucrose cushion. They were devoid of cytoplasmic contaminants as judged by phase contrast microscopy and immunofluorescent staining of isolated nuclei from P19END-2 cells with a monoclonal antibody (TROMA-1) directed against the cytoplasmic endo A cytokeratin (Kemler et al., 1981) (results not shown). Previous work from our group showed that there are marked differences in protein composition of the nuclear matrix among cells in various differentiation states (Stuurman et al., 1989). We examined the protein composition of the peripheral nuclear matrix from a variety of mouse embryonal carcinoma cell lines by two-dimensional NEPHGE-SDS-PAGE and studied the effect of in vitro differentiation of P19EC cells (induced by retinoic acid) on the protein composition of the peripheral nuclear matrix. The results are shown in Fig. 1. The protein composition of peripheral nuclear matrices from the undifferentiated cell line P19EC is, at first view, similar to that of peripheral matrix preparations of P19EC cells induced to differentiate by treatment with retinoic acid for 8 days. On close inspection, however, a number of differences, as indicated by the arrows in Fig. 1, are found. The protein composition of peripheral matrix preparations of P19END-2 (a P19EC derivative) and Dif-5 (a F9 derivative) is even more different.

Apart from proteins that seem to be specific for a certain cell line, a set of matrix proteins is present which is common to all cell lines (see below). Analysis of the peripheral matrix protein composition of PYS-2, P19MES-1, P19EP1-7, F9, PC13, and F9AC c19 (all mouse embryonal carcinoma cell lines) confirmed these observations (not shown).

Protein Composition of the Peripheral Nuclear Matrix from Mouse Tissues and Cells of Other Mammals—In a next step we compared the protein composition of the peripheral nuclear matrix from established mouse cell lines with that of different mouse tissues. We isolated peripheral matrices from mouse liver and brain and analyzed them by two-dimensional NEPHGE-SDS-PAGE (Fig. 2). These experiments confirmed the observations made on peripheral nuclear matrices of tissue culture cell lines. Marked differences in protein composition were observed between peripheral matrix preparations from liver and brain. Only a subset of matrix proteins was common to peripheral matrices from both tissues. The same set was

1 The abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis, NEPHGE, nonequilibrium pH gradient gel electrophoresis, LS, low salt; HS, high salt.
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FIG. 1. Silver-stained two-dimensional NEPHGE-SDS-PAGE of peripheral nuclear matrix preparations from P19EC, P19EC treated for 8 days with retinoic acid (8d.RA), P19END-2, and Dif-5. Peripheral matrices were isolated as indicated under "Materials and Methods," and 50 μg of matrix protein was subjected to two-dimensional gel electrophoresis as described under "Materials and Methods." The molecular mass (in kDa) of marker proteins run on the same gel (second dimension) is indicated on the right side of the figure. The isoelectric points of marker proteins run in the same experiment in a separate tube gel are indicated on the top of the figure. Arrows indicate conspicuous differences in matrix protein pattern between P19EC and P19EC induced to differentiate with retinoic acid for 8 days. Triangles point to three of the common matrix proteins (see "Results") and are to be used as an internal reference.

Also found to be present in mouse spleen (not shown) and kidney (see Fig. 3). Strikingly, this common set of polypeptides was essentially identical to the one found in embryonal carcinoma cell lines.

To investigate whether cells from other species display the same common matrix proteins, we compared the protein composition of the peripheral nuclear matrix from rat liver and human K562 erythroleukemia cells with the protein patterns obtained in the experiments on murine cells. Close inspection of the gels in Fig. 2 shows that a number of matrix proteins that are common to different mouse cells/tissues are shared by the rat and human cells.

These common proteins are schematically depicted in the right panel of Fig. 3. Spots marked La, Lb, and Lc in Fig. 3 were identified by immunoblotting as lamins A, B, and C, respectively (not shown). One spot, designated as nr. 5, was recognized in immunoblotting experiments by a monoclonal antibody (E3) specific for the chicken lamin B2 and rat liver minor lamins2 (Lehner et al., 1986). This protein (lamin D)

2 L. de Jong, unpublished data.

was present in two-dimensional electropherograms from all preparations studied (except for that from Dif-5, although immunofluorescence studies showed that the monoclonal antibody E3 bound to the nuclear lamina of these cells (not shown)). The position of lamin D on two-dimensional electropherograms varied markedly from species to species. Evidently the number of matrix proteins to be designated as common, as depicted in Fig. 3, is a minimum number. This number depends both on the method used to isolate the matrix and on the degree of scrutiny applied in comparing the different gels with each other. As far as the latter is concerned, we have taken care to be very conservative in our estimates.

As expected, the protein composition of the peripheral matrix of mouse kidney is markedly different from that of the postnuclear supernatant from the same tissue (compare the left and middle panels of Fig. 3). Of the 14 common matrix polypeptides, we find two or possibly three corresponding spots on two-dimensional gels of postnuclear supernatant (compare the left and middle panels of Fig. 3). The comigrating proteins correspond to spots numbered 13, 4, and possibly 3 in the right panel of Fig. 3. The exact position of the common matrix proteins was determined by two-dimensional gel electrophoresis of a mixture of equal amounts of protein of peripheral matrix preparation and postnuclear supernatant (not shown) and by comparing the results of these two-dimensional gels with those of the two-dimensional gels of the individual fractions shown in Fig. 3. The apparent overlap in protein composition might indicate that (i) some proteins are located both in the nuclear matrix and in an extranuclear compartment; (ii) a limited number of nonnuclear proteins

FIG. 2. Two-dimensional NEPHGE-SDS-PAGE of peripheral nuclear matrix preparations from mouse liver and brain, rat liver, and human K562 erythroleukemia cells. For further details, see the legend to Fig. 1.
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contaminates the nuclear matrix fraction; or (iii) some matrix proteins fortuitously comigrate with nonnuclear polypeptides. It is clear however that the vast majority of the nuclear matrix proteins, including the common set of peripheral matrix proteins, is different from those in the postnuclear supernatant.

Protein Composition of the Internal Nuclear Matrix—Matrices isolated under reducing conditions are considered to consist mainly of peripheral nuclear matrix proteins (cf. Kaufmann et al., 1981, 1983). Therefore, it is important to determine whether the internal nuclear matrix subfraction also contains a set of common proteins. Matrices derived from nuclei stabilized by oxidation with sodium tetrathionate contain a higher percentage of the original nuclear protein than matrices isolated in the presence of reducing agents (Kaufmann et al., 1981). We isolated these disulfide bond-stabilized matrix proteins, which, according to Kaufmann and Shaper (1984), make up the internal nuclear matrix. The protein composition of this fraction (see Fig. 4) is quite different from that of the postnuclear supernatant (compare Figs. 3 and 4). However, comparison of the internal matrix proteins from mouse liver and mouse brain shows that this fraction too contains proteins common to both tissues (Fig. 4, indicated by triangles) as well as tissue-specific proteins (Fig. 4, indicated by arrows). The internal nuclear matrix proteins shared by mouse liver and brain are not identical to the common matrix proteins indicated in Fig. 3 (compare Figs. 3 and 4).

DISCUSSION

The composition of the nuclear matrix, as judged by two-dimensional gel electrophoresis, is of an astonishing complexity. In this report, we provide evidence that several nuclear matrix proteins, identified by their isoelectric point and molecular weight, are shared by murine cells of various differentiation stages. Lamin B, known to be expressed throughout differentiation (Guilly et al., 1987; Worman et al., 1988; Paulin-Lavasseur et al., 1988), is part of this common set. Lamins A and C are not because they are absent from undif-
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differentiated embryonal carcinoma cells (Lebel et al., 1987; Stewart and Burke, 1987; Stuurman et al., 1989). At least a high proportion of these murine common proteins is also present in rat and human cells, indicating evolutionary conservation of these proteins. Since these polypeptides constitute a group of matrix proteins that occur in matrices of all cells analyzed thus far, we call them minimal matrix proteins.

What may be the interpretation of such a set of minimal matrix proteins? Evidently, minimal matrix proteins are found in all mouse cell types and tissues and in cells from other mammalian species (rat, human). Most likely, their function is the same in all different cells. They probably fulfill a variety of indispensable nuclear functions. The minimal matrix proteins may represent structural, enzymatic, as well as regulatory entities. Many nuclear processes such as RNA synthesis, DNA replication, and spatial organization of chromatin are found associated with the nuclear matrix (for reviews, see Nelson et al., 1984; Nigg, 1988). Virtually nothing is known about the matrix proteins that are involved in these processes.

The minimal matrix proteins may play a role in differentiation. In this study, two methods to isolate nuclear matrix fractions, as analyzed by two-dimensional gel electrophoresis, is completely different. Importantly, in both fractions a set of common proteins is found. These two differentiation stages (Schliwa, 1986), we expect the same to hold for the minimal matrix proteins. Moreover, the procedure used in isolating the nuclear matrix tends to select for structural proteins.

It must be stressed that the current set of minimal matrix proteins represents an underestimate of the true number of common proteins. In our approach, only proteins with an isoelectric point and molecular weight that do not change, for instance during differentiation, are classified as minimal matrix proteins. A difference in post-translational modification of a matrix protein among different cells, for example, would exclude such a protein from the current set of minimal matrix proteins.

In this study, two methods to isolate nuclear matrix fractions were employed, one resulting in an enrichment of peripheral nuclear matrix proteins, the other in an enrichment of internal matrix proteins. In agreement with the data provided by Kaufmann and Shaper (1984), the protein composition of these two fractions, as analyzed by two-dimensional gel electrophoresis, is completely different. Importantly, in both fractions a set of common proteins is found. These two sets are not overlapping. We speculate that proteins from both sets play important roles in nuclear organization.

Apart from common proteins we also find numerous differences in the protein composition of nuclear matrices isolated from different cells and tissues. This finding is in agreement with the data provided by Fey and Penman (1987, 1988). Since these differences are only based on differences in isoelectric point and molecular weight, it is not clear whether they are accounted for by entirely different proteins, by major differences in post-translational modification, or by different proteins with a limited degree of homology. An example of this last category is the putative lamin D, which has a different isoelectric point and molecular weight in different species but a common antigenic epitope, since the protein is specifically recognized in several species by the same monoclonal antibody.

Future research will focus on the function of nuclear matrix proteins. The identification of a minimal matrix as described in this report is helpful in selecting those nuclear matrix proteins that have an important function in nuclear organization.

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