Nuclear Matrix Association of Multiple Sequence-Specific DNA Binding Activities Related to SP-1, ATF, CCAAT, C/EBP, OCT-1, and AP-1†

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ABSTRACT: The association of DNA binding proteins with the nuclear matrix may be related to a functional role of this subcellular structure in chromatin organization and gene regulation. In this study, nuclear matrix preparations from human HeLa S3 cervical carcinoma and rat ROS 17/2.8 osteosarcoma cells were assayed for the presence of DNA binding activities using consensus binding sequences of well-characterized transcription factors as probes. Competition analysis shows that each probe interacts with different nuclear matrix proteins in a sequence-specific manner and that DNA binding activities related to or identical with SP-1, ATF, CCAAT, C/EBP, OCT-1, and AP-1 are present in the nuclear matrix fraction of different cell types. Comparison of the relative abundance of these transcription factor binding activities in nuclear matrix and nonmatrix nuclear fractions suggests that the distribution between these two fractions is cell type specific, cell growth dependent, or independent of these biological parameters. These results are consistent with the postulated role of the nuclear matrix in transcriptional regulation of gene expression.

The nuclear matrix is a morphologically and biochemically complex molecular lattice that may contribute to chromatin organization and gene regulation (Berezney, 1991; Pienta et al., 1991; Fey et al., 1991; Jackson, 1991; Freeman & Garrard, 1992). The matrix is composed of the peripherally located lamina/pore complex and the internal nuclear matrix, which contains ubiquitous proteins (e.g., matrins) (Nakayasu & Berezney, 1991; Hakes & Berezney, 1991), heterogeneous nuclear RNAs (Fey et al., 1986; He et al., 1990), and many low-abundance proteins. Nuclear matrix protein composition is at least in part cell type specific (Fey & Penman, 1988; Stuurman et al., 1990; Getzenberg et al., 1991) or developmentally regulated (Stuurman et al., 1989; Dworetzky et al., 1990; Pienta et al., 1992). Assessing the complexity of nuclear matrix composition and identification of intrinsic biochemical activities is required to understand involvement in cell structure and control of gene expression.

Different structural and functional activities have been shown to be associated with the nuclear matrix, including type II DNA topoisomerases (Berrios et al., 1985; Sperry et al., 1989), histone deacetylase (Delcuve & Davie, 1991), viral regulatory proteins (Schirmbeck & Deppert, 1987; Mann, 1990; Schaack et al., 1990; Greenfield et al., 1991), steroid hormone receptors (Barrack, 1987; Metzger et al., 1991; Schuchard et al., 1991), and oncogene-encoded nuclear proteins (Klempnauer, 1988; Waitz & Loidl, 1991). Also, several DNA binding factors have been characterized (Romig et al., 1992; Luderus et al., 1992; Dickinson et al., 1992) that bind to dA/dT-rich DNA sequences typically found in matrix attachment regions (MARs) (Gasser & Laemmli, 1986; Cockerill & Garrard, 1986). Apart from these DNA binding proteins with limited sequence specificity, two DNA binding activities have been described that recognize short specific consensus elements (NMEs) resembling transcription factor binding sites. These proteins, designated NMP-1 (Dworetzky et al., 1992) and NMP-2 (Bidwell et al., 1993), recognize respectively specific consensus binding sequences related to transcription factors ATF and C/EBP.

These findings raise the possibility that transcription factors are present in the nuclear matrix and that this localization may be fundamental to the postulated role of the nuclear matrix in gene regulation. To address this, we have monitored nuclear matrix protein preparations from several cell types for the presence of sequence-specific DNA binding proteins that are related to transcription factors playing pivotal roles in both developmental and constitutive regulation of gene expression (Mitchel & Tjian, 1989). Our key observation is that a broad spectrum of these factors appears to associate selectively with the nuclear matrix, which may reflect functional properties of the cell.

MATERIALS AND METHODS

Protein Preparations. Nuclear matrix proteins were isolated as described previously from confluent rat ROS 17/2.8 osteosarcoma cells and from continuously proliferating human HeLa S3 cervical carcinoma cells (Bidwell et al., 1993). Proteins obtained by 0.42 M KCl extraction of isolated nuclei, operationally defined here as nonmatrix nuclear factors, were isolated by standard procedures (Dignam et al., 1983) as described previously (van Wijnen et al., 1991b; van den Ent et al., 1993). Quantitation of proteins was performed by Bradford analysis (Pierce).

Gel Shift Analysis. Oligonucleotides used as probes in gel shift assays are summarized in Figure 1. Probes were prepared as described previously (van Wijnen et al., 1992) by labeling with ^{32}P using T4 polynucleotide kinase. Binding reactions were performed similarly as described (Bidwell et al., 1993) in a 20-μL volume with 10 ng/μL poly(dI-dC)·poly(dI-dC), 15% glycerol, 75 mM KCl, 16 mM Hepes, pH 7.5, 0.15 mM EDTA, and 10 fmol of probe DNA. All binding mixtures

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contained 1.0 μg of nuclear protein from matrix or nonmatrix preparations, unless indicated otherwise. Electrophoretic fractionation was performed in 5% (80:1) polyacrylamide gels in TGE buffer (Ausubel et al., 1987), followed by autoradiographic exposure.

Competition analysis was performed by addition to the binding reaction of a 100-fold molar excess (50 nM; 10–30 ng) of either specific competitor DNA or an unrelated oligonucleotide of similar size. Super shift experiments were performed by preincubation of protein samples with commercially available antibody preparations for 15 min at room temperature. Quantitation of protein/DNA interactions was done by image analysis (GDS2000 Gel Documentation System, Ultra-Violet Products Inc., San Gabriel, CA) of short autoradiographic exposures.

RESULTS

To understand the representation of transcription factors in the nuclear matrix in relation to structural and functional properties of the cell, we have compared nuclear matrix protein preparations (Fey & Penman, 1988) with standard transcription factor preparations (0.42 M KCl salt extract of isolated nuclei) (Dignam et al., 1983) for the presence of DNA binding activities. Transcription factor preparations consist of numerous low-abundance non-histone chromatin proteins, including many gene-specific transcription factors, as well as RNA polymerase II and associated proteins, but apparently contain negligible amounts of core histones and other structural nuclear proteins. Nuclear matrix proteins are isolated by solubilization of nuclear matrices that were obtained by sequential detergent extraction and extensive DNase I digestion of cells in situ using a procedure maintaining cellular morphology (Fey & Penman, 1988). Here, proteins present in 0.42 M KCl nuclear extracts are operationally defined as nonmatrix nuclear factors. A priori, this definition reflects neither complementarity nor overlap in composition of this protein population with the composition of nuclear matrix protein preparations. However, the results presented in this study indicate that these preparations represent two distinct subsets of nuclear proteins.

DNA Binding Activities Related to Transcription Factors SP-1 and ATF Are Present in Nuclear Matrix and Nonmatrix Preparations. Transcription factor SP-1 is a general ("housekeeping") trans-activating protein that stimulates RNA polymerase II transcription at a broad spectrum of promoters (Mitchel & Tjian, 1989) by recognition of the high-affinity element 5′dGGGGCGGGGC and a series of related sequences designated GC boxes. The ATF superfamily of transcription factors is represented by a series of proteins (ATF-1, ATF-2, etc.) that form homo- and heterodimers recognizing 5′dTGACG and flanking sequences. We prepared radiolabeled DNA oligonucleotides containing high-affinity SP-1 and ATF binding sites (Figure 1). Binding of nuclear matrix proteins to these probes was compared with interactions mediated by nonmatrix nuclear factors using protein preparations derived from rat osteosarcoma (ROS 17/2.8) and human cervical carcinoma (HeLa S3) cells (Figure 2). Sequence specificity of these interactions is evidenced by competition with specific SP-1 (Figure 2A) and ATF (Figure 2B) oligonucleotides but not with unrelated DNA fragments. The results clearly indicate that SP-1 and ATF binding activities are not only found in nonmatrix nuclear fractions but also are observed in nuclear matrix preparations from both ROS 17/2.8 and HeLa S3 cells. Similar results were obtained with human MG-63 and rat UMR106 osteosarcoma cells, as well as rat H4 hepatoma cells for SP-1 (data not shown) and the ATF-like factor NMP-1 (Dworetzky et al., 1992; Bidwell et al., 1993). Taken together, these results suggest that SP-1 and ATF binding activities are associated with the nuclear matrix of several cell types.

SP-1	5′-CGGA**TGGGCGGGGC**CGGGGA**TGGGCGGGGC**CGG-3′
ATF	5′-CGGAAA**AGAAATGACGAAA**TGTCGAGA-3′
CCAAT	5′-TCACATATAT**GGACCAATCCAA**GAGGG-3′
C/EBP	5′-TGC**TTTAGTGGTTT**TTCGG-3′
OCT-1	5′-AACTCTTCACC**TTATTTGCATAA**GCGATTCTACTGCTC-3′
AP-1	5′-CG**TGACTCA**GCGCGCG-3′

FIGURE 1: Summary of oligonucleotides used for detection of transcription factors. Shown is the double-stranded portion of each DNA fragment, with most oligonucleotides having a 5′dGATC or similar protruding overhang. Specific consensus binding sequences for each factor are underlined. The high-affinity SP-1 and AP-1 binding sites are based on consensus sequences (Mitchel & Tjian, 1989). The ATF (histone H4) (van Wijnen et al., 1989), CCAAT (histone H3) (van Wijnen et al., 1991a), OCT-1 (histone H2B) (Fletcher et al., 1987), and C/EBP (osteocalcin) (Bidwell et al., 1993) binding sites were synthesized according to the natural sequences present in the promoters of the indicated genes.

The Presence of DNA Binding Activities Related to CCAAT and C/EBP Transcription Factors in the Nuclear Matrix Is Cell Type Specific. We also performed gel shift assays with binding sites for CCAAT- and C/EBP-related transcription factors which, similar to SP-1 and ATF elements, are present in the 5′ regulatory regions of many genes. Figure 3A shows that nuclear matrix proteins interacting with a CCAAT motif are very abundant in preparations of HeLa S3 but not of ROS 17/2.8 cells, whereas identical to previous results (Bidwell et al., 1993), proteins binding to a C/EBP motif are more abundant in nuclear matrices of ROS 17/2 than HeLa S3 cells (Figure 3B). The results presented in Figure 3 indicate that the apparent abundance of CCAAT and C/EBP transcription factors in the nuclear matrix is cell type specific.

Differences in the relative abundance of several DNA binding activities per microgram of protein from nuclear matrix and nonmatrix preparations (Figures 2 and 3; see also Figure 5 below) suggest that these preparations represent two distinct sets of nuclear proteins, although these groups may partially overlap. The abundances of SP-1 and ATF are similar in both matrix and nonmatrix nuclear fractions, with these factors having slightly lower specific activities in nuclear matrix samples from each cell type (Figure 2). In contrast, for example, the specific activity of CCAAT box binding proteins is severalfold higher in the matrix fraction (Figure 3A) than in the nonmatrix fraction of HeLa S3 cervical carcinoma cells but is barely detectable in ROS 17/2.8 osteosarcoma cells. This finding suggests that these CCAAT box binding activities are preferentially associated with the nuclear matrix in cervical (epithelial) carcinoma but not osteosarcoma cells. Similarly, as shown previously (Bidwell et al., 1993), the C/EBP-related factor NMP-2 (a 38-kDa bone cell-related protein) is very abundant in nuclear matrices of osteosarcoma cells but is not or is barely detectable in nonmatrix fractions (Figure 3B). Direct comparison of nuclear protein distribution in Figures 2 and 3 indicates that CCAAT- and C/EBP-related factors are both nuclear matrix specific and distinctively cell type dependent, whereas SP-1- and ATF-related proteins are cell type independent and are equally represented in matrix- and nonmatrix-derived protein populations (see Figure 5 below for summary).

Association of the Cell Growth Regulated Transcription Factors OCT-1 and AP-1 with the Nuclear Matrix. OCT-1 binding activity is a cell growth regulated POU/homeodomain transcription factor (Fletcher et al., 1987) that is abundant in nuclear extracts of continuously proliferating HeLa S3 cells

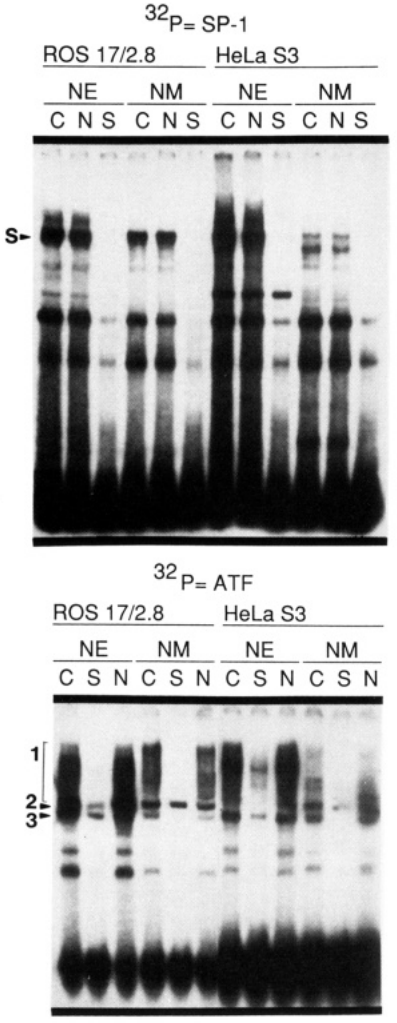

FIGURE 2: Presence of SP-1 and ATF binding activities in nuclear matrix and nonmatrix preparations. Gel shift analysis of SP-1- (panel A, top) and ATF- (panel B, bottom) related DNA binding activities isolated from ROS 17/2.8 rat osteosarcoma cells (from left, lanes 1–6) or HeLa S3 human cervical carcinoma cells (lanes 7–12). Binding reactions were performed with 1.0 μg of nonmatrix (NE) (lanes 1–3 and 7–9) and nuclear matrix (NM) derived proteins (lanes 4–6 and 10–12). Specificity of binding was established by competition using control binding reactions (C = lanes 1, 4, 7, and 10) that were performed in the absence of specific unlabeled competitor DNA or binding reactions containing a 100-fold molar excess of specific unlabeled probe DNA (lanes designated S) or an unrelated nonspecific oligonucleotide of similar size (lanes designated N). The arrowheads and capitals indicate the main binding activities observed for each probe, respectively SP-1 (bold S in panel A) and ATF (bold numbers **1**, **2**, and **3** in panel B; complex **1** refers to a series of ubiquitous ATF proteins that are unquestionably specific, but complexes **2** and **3** appear to comigrate to various degrees with nonspecific binding activities in each preparation, i.e., residual complexes observed in lanes 2, 5, 8, and 11). Undesignated complexes observed for these and other probes may represent different posttranslational modifications of single binding activities (including phosphorylation, glycosylation, and protein/protein associations), reflect minor members of each transcription factor family, or involve noncompetable binding activities.

(Figure 4A). Similar to observations with SP-1- and ATF-related DNA binding proteins, the specific activity of OCT-1 is similar in both matrix and nonmatrix nuclear fractions of HeLa S3 cells. OCT-1 activity was also observed with nuclear matrix proteins from ROS 17/2.8, MG-63, UMR-106, and H4T cells (data not shown). The similar distribution of SP1, ATF, and OCT-1 binding activities over two distinct (matrix versus nonmatrix) protein populations (Figures 2 and 4) suggests that this may be a common property of these, and perhaps other, ubiquitous transcription factors.

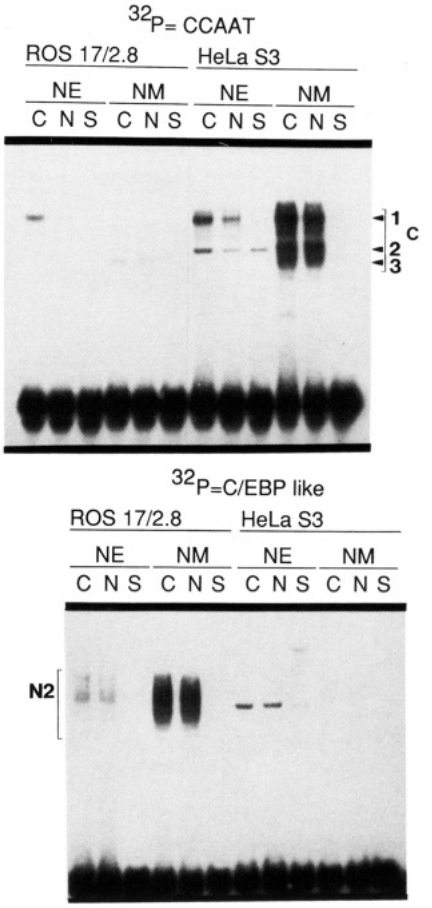

FIGURE 3: Cell type dependent relative abundance of CCAAT and C/EBP binding activities in nuclear matrix preparations. Binding reactions and competition analysis were performed as explained in detail in Figure 2. The CCAAT oligonucleotide (panel A, top) detects three prominent specific complexes (designated **1**, **2**, and **3**). Complex **1** is mediated by the heteromeric CCAAT box binding protein HiNF-B (a member of the CP-1/NF-Y type of CCAAT box transcription factors) (van Wijnen et al., 1991a), complex **2** appears to comigrate with both nonspecific (e.g., lanes 7–9) and specific DNA binding activities (e.g., lanes 10–12), whereas complex **3** is a higher mobility specific complex detected primarily with nuclear matrix proteins (lanes 4–6 and 10–12). The C/EBP oligonucleotide (panel B, bottom) detects a series of DNA binding activities (indicated by the bracket and abbreviation N2) that are particularly abundant in the nuclear matrix of osteosarcoma cells (lanes 4–6), and the main DNA binding factor unique to this fraction is a 38-kDa protein designated NMP-2 (Bidwell et al., 1992). Additional C/EBP-like proteins with different mobilities (molecular mass ranging from 32 to 42 kDa) are observed in the nonmatrix nuclear fraction of both ROS 17/2.8 (lanes 1–3) and HeLa S3 (lanes 4–6) cells.

AP-1 is a heterodimeric class of transcription factors composed of FOS- and JUN-related proteins. AP-1 binding activity is similarly abundant in both the nonmatrix and nuclear matrix fractions of HeLa S3 cells (Figure 4B), but this distribution is strikingly different in ROS 17/2.8 cells (Figure 4C) where there is more AP-1 binding activity in the nuclear matrix fraction. For example, using the same amount of protein (1 μg), AP-1 is barely detectable in the nonmatrix nuclear fraction of confluent ROS 17/2.8 cells (Figure 4C), consistent with the cell growth related downregulation of AP-1 in these cells at confluency (van den Ent et al., 1993), but is clearly present in the nuclear matrix. Using a 5-fold higher amount of protein (5 μg), it is possible to detect AP-1 binding in nonmatrix preparations from confluent ROS 17/2.8 cells (Figure 4B), but the amount of AP-1 detected is still less than that obtained with 1 μg of nuclear matrix protein from ROS 17/2.8 or HeLa S3 cells. We have assayed the same preparations also for the presence of other DNA binding

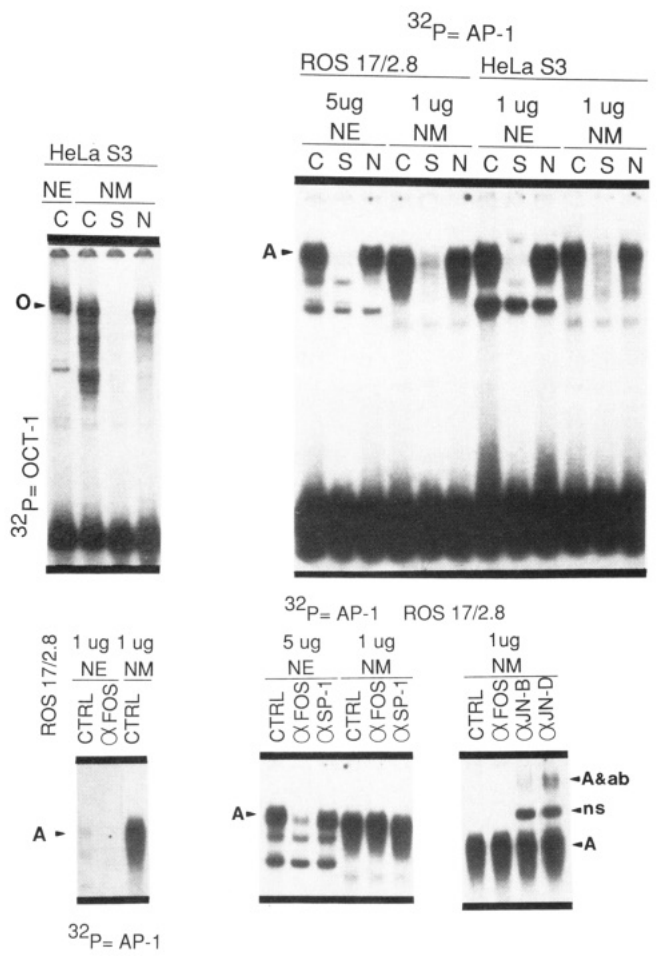

FIGURE 4: Presence of cell growth regulated transcription factors OCT-1 and AP-1 in matrix and nonmatrix nuclear preparations. Panels A and B (top left and top right, respectively) show binding reactions and competition analysis that were performed as explained in Figure 2. Factor OCT-1 observed in HeLa S3 cells is shown in panel A (indicated with bold **O**), and AP-1 binding activity representing a series of FOS- and JUN-related proteins is shown in panel B (indicated with bold **A**). Lanes 1–3 in panel B are the only binding reactions in this study in which 5 μg instead of 1 μg of protein is added to show specific competition of low levels of AP-1 activity present in confluent ROS 17/2.8 cells. Panel C (bottom, left portion) shows binding reactions performed with in each case 1 μg of protein of nonmatrix (lanes 1 and 2) and matrix nuclear (lane 3) fractions from ROS 17/2.8 cells in the absence (lanes 1 and 3) or presence (lane 2) of a FOS antibody (compare intensities of signals in panels B and C). Panel C (middle and right portions) shows binding reactions with nonmatrix and matrix nuclear protein in the absence (lane CTRL) or presence of antibodies (abbreviated ab) directed against FOS, JUN-B, JUN-D, or SP-1 (nonspecific control). The band labeled ns (right panel) represents a nonspecific complex that did not increase in proportion with nuclear protein quantity.

activities (summarized in Figure 5), which provides internal controls for the integrity and quantity of nuclear proteins. Taken together, our results indicate that although AP-1 is downregulated in the nonmatrix nuclear fraction (presumably representing primarily chromatin-associated proteins) when ROS 17/2.8 cells cease proliferation (van den Ent et al., 1993), a substantial amount of AP-1 binding activity is present in the nuclear matrix in these cells.

The distribution of AP-1 activity over the matrix and nonmatrix fractions in continuously proliferating HeLa S3 cells is very different from that of confluent ROS 17/2.8 cells, suggesting that this distribution is cell growth related. However, the differential distribution of nuclear proteins in these two cell types appears to be primarily due to low levels of AP-1 in the nonmatrix fraction of ROS 17/2.8 cells, while the levels of AP-1 binding activity in the nuclear matrix of

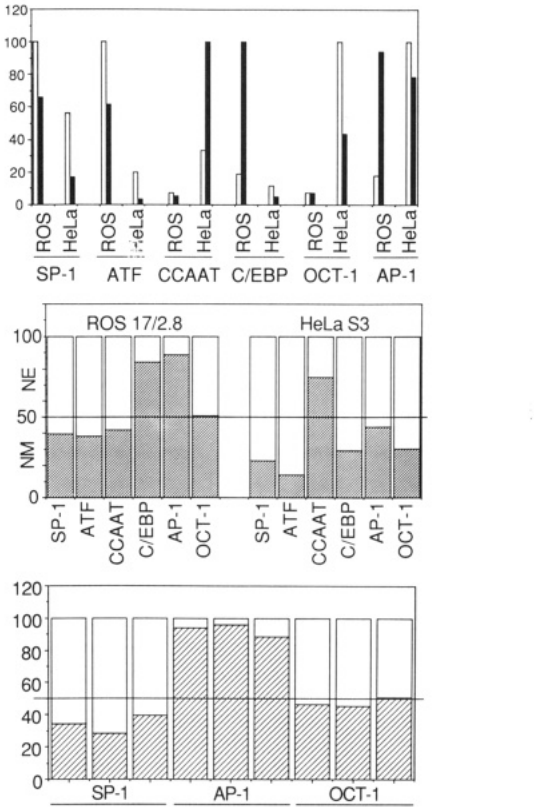

FIGURE 5: Cell type and cell growth dependent differences in the specific activity of transcription factors in matrix and nonmatrix nuclear preparations. (A, top) Quantitation of transcription factor binding activities obtained by analyzing the relative intensity of gel shift complexes (indicated vertically and expressed as arbitrary units) on representative autoradiograms. This graph compares the distribution of the binding activities monitored with the indicated probes (displayed horizontally) using identical amounts of protein (1.0 μg) of matrix (black bars) and nonmatrix (open bars) nuclear fractions from ROS 17/2.8 and HeLa S3 cells (respectively, left and right in each group). Gel shift complexes were initially expressed as densitometric units per microgram of protein and, for each probe, were normalized relative to the most intense band resulting in dimensionless units (percent of maximum value). The most striking observation is the cell-type specificity of relative transcription factor abundance in nuclear matrix preparations (e.g., see CCAAT-, C/EBP-, and AP-1-related activities). (B, middle) Distribution of transcription factor binding activities between matrix (diagonally striped bars; abbreviated NM) and nonmatrix (open bars; abbreviated NE) nuclear fractions of ROS 17/2.8 (left portion) and HeLa S3 cells (right portion). Densitometric units were obtained as described in part A. For each probe, this representation was obtained using the following expression: [percentage activity of a given DNA binding activity in the nuclear matrix] = 100[units of protein/DNA complexes observed with nuclear matrix proteins]/[total units of complexes observed with matrix and nonmatrix nuclear proteins]. In visual terms, the values depicted in panel B were obtained by adding the values for the white and black bars from panel A and expressing the value of each bar as the fraction of this total. The fraction of each DNA binding activity in the nuclear matrix and nonmatrix nuclear preparations is indicated by striped and open bars, respectively. A value close to 50% (horizontal line in graph) implies that the relative abundance of the DNA binding activity is equal in both matrix and nonmatrix preparations. This calculation facilitates standardization of data from several experiments as shown in Figure 4C. (C, bottom) Reproducibility of transcription factor distribution between matrix and nonmatrix nuclear fractions is exemplified by the relative abundance of SP-1, AP-1, and OCT-1 in three distinct experiments with ROS 17/2.8 cells. The relative distribution of these DNA binding activities between the two nuclear fractions was calculated as described in part B (NM fraction, striped bars; NE fraction, open bars). From left, columns 1, 4, and 7 were obtained using values acquired during the same assay; the same is true for columns 2, 5, and 8, as well as columns 3, 6, and 9, respectively. Columns 1, 3, 4, 6, 7, and 9 were determined with the same protein sample and reflect interassay variation, whereas the remaining lanes were obtained with a duplicate protein sample and reflect variation in the preparation of the protein fractions.

HeLa S3 and ROS 17/2.8 cells are comparable (Figure 4). The precise biological conditions that determine the levels of FOS and JUN proteins in the nuclear matrix remain to be established. There may be two populations of AP-1 molecules; for example, AP-1 in the nonmatrix fraction may be cell growth regulated, whereas AP-1 in the nuclear matrix fraction could be constitutively present. Alternatively, there may be an active equilibrium between these two populations. The current results do not discriminate between these and other possibilities, and the functional significance with respect to regulation of AP-1-responsive genes remains to be explored.

The specific presence of AP-1 in the nuclear matrix of confluent ROS 17/2.8 cells may involve distinct members of the AP-1 protein family. To begin addressing this question, we monitored the effect of antibodies against FOS, JUN-B, and JUN-D on AP-1 activity in gel shift assays. Interestingly, the FOS antibody almost quantitatively blocks binding of AP-1 with nonmatrix nuclear proteins, but no effect is observed with nuclear matrix proteins (Figure 4C). In comparison, JUN-B and JUN-D antibodies form ternary complexes with the AP-1 protein/probe complex with both matrix and nonmatrix nuclear proteins. Thus, these results suggest that immunologically detectable differences may exist in matrix and nonmatrix nuclear populations of AP-1 activity. These differences may indicate that the presence of AP-1 in the nuclear matrix involves JUN- but not FOS-related proteins, although alternative explanations are plausible.

DISCUSSION

The nuclear matrix contains ubiquitous proteins (e.g., lamins, matrins) that are common to nuclear matrices from many cell types, as well as low-abundance proteins that are cell type specific (Fey et al., 1991). In this and related studies (Bidwell et al., 1993), we have shown that several DNA binding activities, including proteins related to transcription factors SP-1, ATF, OCT-1, and AP-1, can be detected in both nonmatrix and matrix-specific nuclear fractions. This apparent association of these factors with the nuclear matrix occurs in several cell types. Moreover, we have observed CCAAT- and C/EBP-related proteins that appear to be cell type specific and restricted to the nuclear matrix. This multiplicity and cell stage dependence of DNA binding activities in the nuclear matrix may reflect in part the cell-type specificity and developmental regulation of nuclear matrix protein composition. Our findings are consistent with one of the postulated roles of the nuclear matrix in regulation of gene expression, which involves the selective localization of (a subset of) transcription factors resulting in regional concentration of low-abundance, positive (or negative) trans-acting proteins (Stein et al., 1991). In addition, the nuclear matrix may serve as a reservoir by sequestering regulatory proteins in a dormant state.

This proposed mechanism in concentrating regulatory factors is complementary to, for example, the involvement of the nuclear matrix in determining spatial organization and topology of chromatin by interaction with specific stable matrix attachment regions (MARs) (Pienta et al., 1991; Freeman & Garrard, 1992). Because of the differential presence of some transcription factor DNA binding activities in both nonmatrix and matrix nuclear fractions, it is plausible that these regulatory proteins are subject to dynamic shuttling between distinct subnuclear compartments. In addition, the clustering and distinct permutations of binding sites in many gene promoters raise the possibility that some aspect of gene/nuclear matrix attachment could be intrinsic properties of transcription factors. These concepts can be further defined experimentally.

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