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Subcellular Partitioning of Transcription Factors During Osteoblast Differentiation: Developmental Association of the AML/CBFα/PEBP2α-Related Transcription Factor-NMP-2 With the Nuclear Matrix

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Abstract The subnuclear location of transcription factors may functionally contribute to the regulation of gene expression. Several classes of gene regulators associate with the nuclear matrix in a cell type, cell growth, or cell cycle related-manner. To understand control of nuclear matrix-transcription factor interactions during tissue development, we systematically analyzed the subnuclear partitioning of a panel of transcription factors (including NMP-1/YY-1, NMP-2/AML, AP-1, and SP-1) during osteoblast differentiation using biochemical fractionation and gel shift analyses. We show that nuclear matrix association of the tissue-specific AML transcription factor NMP-2, but not the ubiquitous transcription factor YY1, is developmentally upregulated during osteoblast differentiation. Moreover, we show that there are multiple AML isoforms in mature osteoblasts, consistent with the multiplicity of AML factors that are derived from different genes and alternatively spliced cDNAs. These AML isoforms include proteins derived from the AML-3 gene and partition between distinct subcellular compartments. We conclude that the selective partitioning of the YY1 and AML transcription factors with the nuclear matrix involves a discriminatory mechanism that targets different classes and specific isoforms of gene regulatory factors to the nuclear matrix at distinct developmental stages. Our results are consistent with a role for the nuclear matrix in regulating the expression of bone-tissue specific genes during development of the mature osteocytic phenotype. J. Cell. Biochem. 66:123–132, 1997. © 1997 Wiley-Liss, Inc.

Key words: AML-3; transcription factors; partitioning; osteoblast differentiation

The nucleus is highly specialized and contains a multiplicity of spatially and functionally distinct domains that represent localized sites for DNA replication and gene expression [1–4]. The architecture of the nucleus is supported by the peripheral lamina/pore complex and the internal nuclear matrix. The nuclear matrix is an intricate nucleoprotein network composed of anastomosing 10 nm filaments, which can be clearly visualized by resinless section electron microscopy under conditions maintaining normal nuclear morphology [1]. This component of nuclear matrix architecture provides a scaffold for the higher order organization of chromatin structure [5–7], and is intimately associated with key aspects of gene expression, including mRNA synthesis, processing, and transport.

The non-random spatial complexity of functionally distinct domains in the nucleus [2–4] and the association of gene regulatory factors with the nuclear matrix [8], suggest that physiological control of gene expression requires the stringently controlled presence of proteins at specific locations within the nucleus. Many different classes of gene regulatory proteins, including transcription factors, oncoproteins, tumor suppressors, hnRNPs, viral proteins, and histone-modifying enzymes are associated with the nuclear matrix [8–18]. These findings are consistent with the concept that the nuclear matrix is involved in control of gene expression by facilitating the subnuclear trafficking and

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compartmentalization of regulatory proteins, while regulatory factors associated with the nuclear matrix become concentrated in specific nuclear regions. This model invokes precise molecular interactions between nuclear matrix components and associated proteins, and is strongly supported by the recent identification of a nuclear matrix targeting signal (NMTS) [19]. This NMTS directs the tissue-specific transactivator AML-1B to the nuclear matrix. In contrast, the related factor AML-1 is derived from a mRNA splice variant, which encodes a truncated protein that is incapable of activating transcription, lacks this NMTS, and is not nuclear matrix associated.

The postulated role of the nuclear matrix in gene regulation also necessitates association of structural and regulatory proteins. These interactions of proteins with the nuclear matrix are not static or non-specific, but rather appear to be dynamic and selective. It has been well established that the composition of nuclear matrix proteins is cell type specific [20,21], dependent on the neoplastic phenotype [22–25], and developmentally regulated [26,27]. Furthermore, nuclear matrix composition is responsive to polypeptide growth factors and steroid hormones in different cell types including bone-related and breast cancer cells [28–32]. Most previous studies of nuclear matrix protein composition were based primarily on two-dimensional electrophoretic analyses. Thus, there is limited insight into the identities of proteins that are absent or present in the nuclear matrix under different biological conditions.

Two nuclear matrix proteins, NMP-1 and NMP-2, have been characterized in osteoblasts and were shown to be sequence-specific DNA binding proteins [33]. Recently, NMP-1 was identified as the transcription factor YY-1 [17,34], and NMP-2 has been shown to be immunologically related to the AML class of transactivators [18]. Furthermore, we and others have shown that the association of these and other gene regulatory factors with the nuclear matrix is cell type, cell growth, or cell cycle related [9,10,33]. However, understanding the regulation of transcription factor association with the nuclear matrix during tissue-development necessitates systematic analyses of the subcellular distribution of these proteins during differentiation.

In this study, we defined the subnuclear partitioning of a panel of transcription factors (including NMP-1/YY-1, NMP-2/AML, AP-1, and SP-1) during osteoblast differentiation using biochemical fractionation and gel shift analyses. The principal results are that transcription factors exhibit differential partitioning between nuclear matrix and non-matrix nuclear compartments during osteoblast differentiation. We also demonstrate that association of the AML transcription factor NMP-2 with the nuclear matrix is developmentally upregulated during bone cell differentiation. It appears that the association of transcription factors with the nuclear matrix is dynamic and may be functionally involved in the regulated expression of bone-tissue specific genes during developmental maturation.

MATERIALS AND METHODS

Isolation of Nuclear Matrix Proteins

Normal diploid rat calvarial osteoblasts (ROB) were derived from fetal calvaria and were cultured as adherent cells [35]. Nuclear matrix fractions were prepared as described [20,33] with minor modifications. Briefly, subcellular fractions were prepared from 5×10^8 cells (1 ml packed cell volume) by resuspending the washed cell pellet in 20 ml of CSK buffer (100 mM NaCl, 300 mM sucrose, 10 mM PIPES/pH 6.8, 3 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton X-100), which was complemented just prior to use with a broad spectrum of protease inhibitors (Boehringer Mannheim, Indianapolis, IN) and additives (0.2 mM PMSF, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, 10 μg/ml Trypsin Inhibitor, 2 μg/ml TPCK, 40 μg/ml bestatin, 17 μg/ml Calpain Inhibitor, 1 μg/ml E64, 1.0 mM EGTA, 0.2 mM EDTA, 1 mM DTT, 0.75 mM spermidine, 0.15 mM spermine). Nuclei were collected by centrifugation for 5 min at 2,000 rpm in an IEC-4B centrifuge. The nuclei were extracted with 10 ml of RSB buffer (10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris/pH 7.4, 1% Tween-40, 0.5% sodium deoxycholate) as above. The pellet fraction was then digested with RNase A and DNase I for 20 min at room temperature after resuspension in 20 ml digestion (DIG) buffer (50 mM NaCl, 300 mM sucrose, 10 mM PIPES/pH 6.8, 3 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton X-100, 100 μg/ml DNase I, 50 μg/ml RNase A, 1.2 mM PMSF). Ammonium sulfate (20 ml) was added to 0.25 M and the precipitates collected by centrifugation at 2,000 rpm for 10 min. The nuclear matrix (NM) samples were prepared by further treatment as follows.

The pellet was resuspended in disassembly buffer (8 M urea, 20 mM MES/pH 6.6, 1 mM EGTA, 0.1 mM $MgCl_2$, 1%-2-mercaptoethanol/ 1.2 mM PMSF) and dialyzed against assembly buffer (0.15 M KCl, 25 mM Imidazole-HCl/pH 7.1, 5 mM $MgCl_2$, 0.125 mM EGTA, 2 mM dithiothreitol, 1.2 mM PMSF). The assembled filaments were removed by ultracentrifugation (40,000 rpm in a Ti 70.1 fixed angle rotor for 90 min at 20°C). The supernatant was concentrated using Centricon 10 tubes (Amicon, Beverly, MA) and resuspended in storage buffer (20 mM HEPES/pH 7.9, 100 mM KCl, 0.2 mM EDTA, 0.5 mM DTT, 20% glycerol, 1.2 mM PMSF).

Preparation of Nuclear Extracts

ROB cells were harvested by rapidly suspending adherent cells in ice-cold 1 × PBS using a plastic scraper. Nuclear protein preparations were obtained by lysing cell pellets (1×10^8 cells or approximately 200 to 300 µl wet packed cell volume) in 4 ml ice-cold buffer R (bR) (10 mM KCl, 10 mM HEPES/pH 7.5, 0.5% Triton, 300 mM sucrose, 3 mM $MgCl_2$), which was complemented with the cocktail of protease inhibitors and additives described above. Cells were mechanically lysed on ice in a homogenizer tube using a Teflon pestle attached to an electric drill. After micro-centrifugation of the lysates at 1,500 rpm using a low speed swing-out centrifuge (IEC-4B), nuclear pellets were resuspended in 1,000 µl buffer A (bA) (10 mM HEPES/pH 7.5, 10 mM KCl) plus the above additives. Samples were transferred to 1.5 ml mifrofuge tubes and subjected to centrifugation at 6,000 rpm for 1 min. The nuclear pellets were extracted with 400 µl buffer C (0.4 M KCl, 25 mM HEPES/pH 7.5, 25% glycerol plus protease inhibitors and additives) for 30 min on ice. Nuclear homogenates were centrifuged for 10 min at 4°C and the supernatant (nuclear extract) was frozen in liquid nitrogen without dialysis. The concentration of nuclear proteins was determined by Coomassie Blue staining using a multiwell plate reader. Absorbance readings were obtained in duplicate for a range of volumes for each sample, and the protein concentrations were calculated using bovine serum albumin as the external protein standard.

Protein/DNA Interaction Assays

The DNA binding activities of transcription factors were monitored with a series of consensus oligonucleotides as described previously [9,18]. Standard binding reactions (20 µl volume) for each factor were performed at room temperature and contain 10 fmole (= 0.2 ng) ^{32}P-labelled DNA probe, non-specific competitor DNAs (0.2 µg poly I/C DNA), and 1 µg protein. Competition assays were performed in the presence of 100-fold molar excess (1 pmole) of the unlabelled DNA probe (self-competition) or an unrelated oligonucleotide of similar size (non-specific competition). Gel shift immunoassays were performed by pre-incubating antibodies with protein on ice for 15 min prior to the addition of probe DNA. Unrelated immunesera (e.g., E2F) were used as control reagents. The polyclonal rabbit antisera against AML-1, AML-2, and AML-3 are subtype-specific. Electophoretic fractionation of protein/DNA complexes was performed in 4% (80:1) polyacrylamide gels using 0.5 × TBE as buffer [36].

RESULTS

Differential Partitioning of YY-1, AP-1, SP-1, and AML-Related Factors in Distinct Subcellular Compartments

To assess the subcellular distribution of transcription factors, we performed gel shift assays and analyzed the DNA binding activities of YY-1, AML, AP-1, and SP-1 in distinct fractions obtained during preparation of nuclear matrices from differentiated osteoblasts (Fig. 1). This procedure involves sequential extraction with detergents and nuclease digestion, and yields four soluble fractions designated CSK, RSB, DIG, and NMP. For comparison with the nuclear matrix fractionation protocol, cells were also subject to nuclear salt-extraction, which yields two cytoplasmic fractions (bR and bA), one nuclear extract (NE) fraction, and a residual nuclear pellet fraction. The residual nuclear fraction is an insoluble pellet that could not be analyzed for DNA binding activity. Fractionation of proteins by the nuclear salt-extraction procedure does not reveal major differences in the relative distribution of YY-1, AML, AP-1, and SP-1 per microgram protein (Fig. 1). As expected for transcription factors, levels of these four proteins are relatively low in two cytoplasmic (bR and bA) fractions, and generally higher in the nuclear extract (NE) fraction.

The CSK fraction contains soluble cytoplasmic components, which are extracted with 100 mM NaCl and 0.5% Triton. This fraction contains low levels of YY-1, SP-1, and AML related

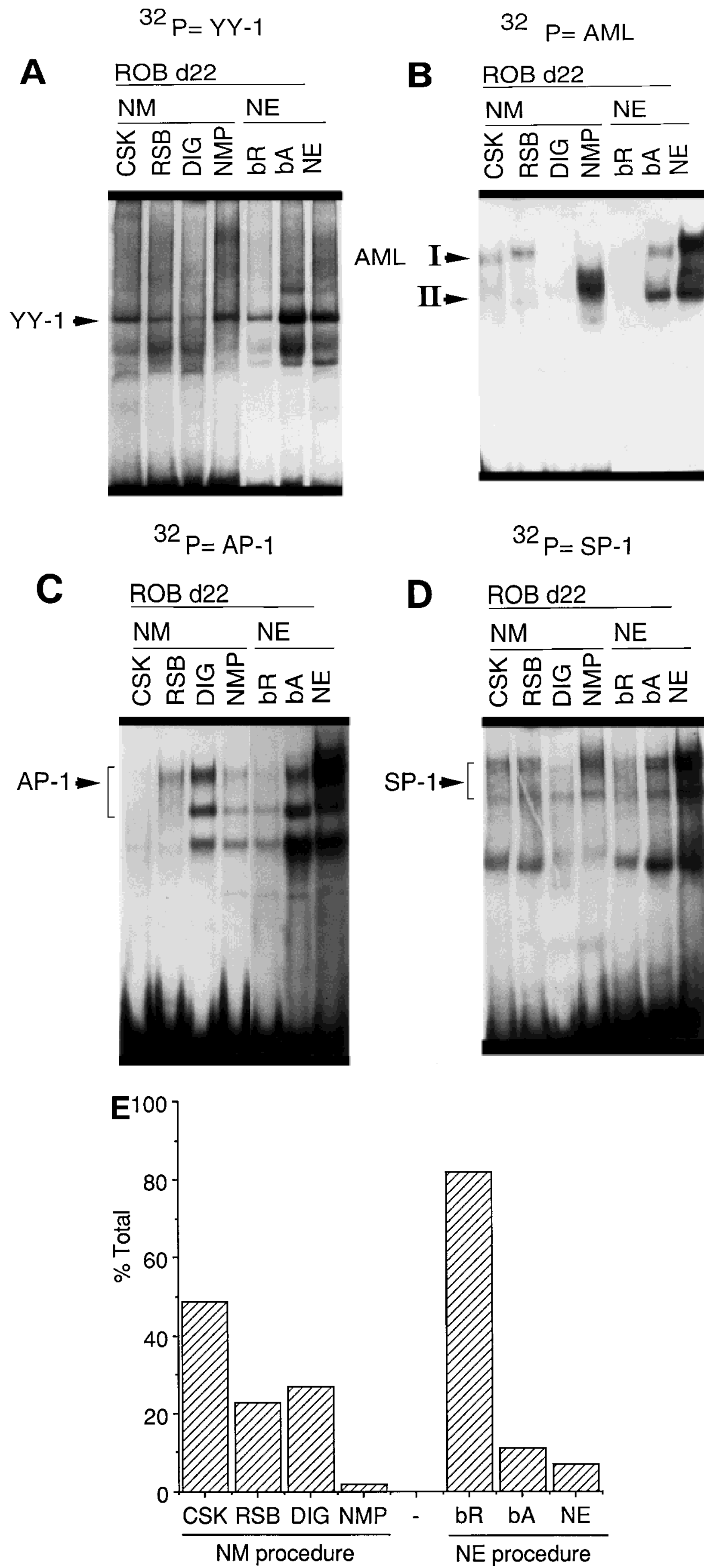

Figure 1.

proteins (Fig. 1A,B, and D), but appears to be devoid of AP-1 factors (Fig. 1C). The extraction of cells with RSB buffer solubilizes cytoplasmic and nuclear proteins in the presence of two detergents (1% Tween and 0.5% sodium deoxycholate), which permeabilize the nuclear membrane. All four transcription factors can be detected in the RSB fraction (Fig. 1). The DIG fraction contains nuclear factors that are released from the remaining nuclear matrix/intermediate filament network by nuclease digestion and salt-extraction with 0.25 M $(NH_4)_2SO_4$. The DIG fraction represents a non-matrix nuclear compartment and contains factors tightly associated with chromatin. Consistent with this concept, the DIG fraction contains relatively high levels of AP-1 activity and detectable levels of SP-1 and YY-1 (Fig. 1A,C, and D). However, the DIG fraction appears to be almost devoid of AML related proteins (Fig. 1B). AML factors are highly enriched in the soluble nuclear matrix protein fraction (NM), in which YY-1, AP-1, and SP-1 proteins are present at levels comparable to those observed in other fractions. Taken together, the data obtained by the nuclear matrix fractionation procedure suggest that at least four different classes of transcription factors (AML, YY-1, AP-1, and SP-1) partition between distinct cytoplasmic and nuclear compartments.

The gel shift assays in Figure 1 were performed with approximately equal protein to reflect the apparent specific activity of transcription factor DNA binding levels in different subcellular fractions. We calculated the actual amount of protein recovered in each fraction and evaluated the relative amount of protein per fraction (Fig. 1E). We compared these values with the specific activity of each transcription factor as determined by densitometric scanning of autoradiograms (data not shown). The results of this analysis reveals for YY-1, which partitions in both nuclear matrix and non-nuclear matrix fractions, that although its specific activity is substantially higher in nuclear matrix fractions than in other fractions obtained during the isolation procedure, the relative amount of YY-1 bound to the nuclear matrix is only a small percentage (<1%) of total YY-1 present in the cell. In contrast, the nuclear matrix specific AML-(II) complex is mediated by an AML protein, which resides exclusively (>90%) in the nuclear matrix, although this protein represents only a small subset of the total AML binding activity detected in all fractions. This finding may suggest fundamental differences in the mechanism by which the nuclear matrix sequesters different classes of nuclear matrix-associated gene regulatory proteins.

Fig. 1. Differential partitioning of YY-1, AP-1, SP-1, and AML-related factors in distinct subcellular compartments. The subcellular distribution of DNA binding activities mediated by YY-1, AML, AP-1, and SP-1 was determined using gel shift assays with soluble fractions (CSK, RSB, DIG, and NMP) obtained during preparation of nuclear matrices (NM procedure, left lanes in each panel) from differentiated osteoblasts (ROB d22), as well as with cytoplasmic (bA and bR) and nuclear extract (NE) fractions obtained by salt-extraction of nuclei (NE procedure, right lanes in each panel). Gel shift assays were performed with radio-labelled probes spanning consensus binding sites for YY-1 **(A)**, AML **(B)**, AP-1 **(C)**, and SP-1 **(D)** using equal amounts of protein (1 μg) for each fraction. Relevant protein/DNA complexes for each probe are indicated by arrowheads and/or brackets. The bracket for AP-1 refers to several complexes mediated by distinct members of the fos/jun family. The bracket for SP-1 refers to three complexes: the complex with lowest mobility is mediated by SP-1, whereas the remaining two are SP-3 related. The designations AML-I and AML-II refer to the most prominent bands observed in, respectively, the NE and NM sample. However, these bands are in fact each composed of several different AML subcomplexes (see Fig. 2). **E:** Relative distribution of soluble protein for each fraction (expressed as a percentage of total soluble protein) obtained during the nuclear matrix and nuclear extract fractionation procedures of representative experiments. Soluble protein (mg) for each fraction was calculated by multiplying the volume (ml) of each fraction with the protein concentration (mg/ml). The range of protein concentrations for different subcellular fractions was comparable (between 0.5 and 1.2 mg/ml).

Multiple Isoforms of the AML Class of Tissue-Specific Transcription Factors Partition in Distinct Subcellular Compartments

As previously reported [18,37], there are at least two different gel shift complexes mediated by the AML class of factors. One complex is mediated by an AML factor present in nuclear extracts (NE) [37], which we refer to in this study as the low mobility AML complex (AML-I). The second complex (AML-II) is mediated by nuclear matrix (NM) proteins and displays a higher electrophoretic mobility [18]. Evaluation of the signals for these two variants in nuclear matrix and non-matrix nuclear fractions confirms that the subcellular distribution of the AML-I and AML-II variants differs (Fig. 1B). However, we noted that the AML-I and AML-II complexes observed in the NE and NM fractions are subdivided in distinct but closely co-migrating subcomplexes, which appear to separate into different subcellular fractions. In-

terestingly, AML proteins are encoded by three different genes (AML-1, AML-2, and AML-3), which each may generate multiple distinct AML proteins [38-40]. Thus, the possibility arises that the subtle differences in the electrophoretic mobilities of AML related gel shift subcomplexes reflect binding of distinct AML proteins derived from alternatively spliced cDNAs and/or different AML genes.

To assess whether each of the gel shift subcomplexes detected with the AML probe has intrinsic AML binding activity, we performed gel shift oligonucleotide-competition assays with the AML consensus oligonucleotide. Figure 2 clarifies the subdivision of the AML-I and AML-II protein/DNA interactions in subcomplexes with different electrophoretic mobilities (designated c1 to c7). Subcomplexes c2 and c3 are present in the CSK fraction, subcomplexes c1 and c4 are predominantly present in the RSB fraction, whereas subcomplexes c5, c6 and c7 are present primarily in the nuclear matrix fraction (Fig. 1B), although low levels of these complexes can be detected in the DIG fraction (Fig. 2A). Specific competition with the AML consensus oligonucleotide reveals that these subcomplexes are indeed mediated by AML related DNA binding activities (Fig. 2A).

To address whether the AML subcomplexes are mediated by AML isoforms derived from different AML genes, we performed gel shift immuno-assays with antibodies against AML-1, AML-2, and AML-3 proteins (Fig. 2B). The results show that complete immuno-reactivity is only observed for subcomplexes c1 and c2 in the presence of the AML-3 antibody as reflected by a loss of signal. In contrast, the other subcomplexes (c3 to c7) detected with ROB proteins are not inhibited in the presence of the AML-1, AML-2, or AML-3 antibodies. We conclude that the identification of two distinct AML-3 containing subcomplexes (c1 and c2) and multiple subcomplexes with discrete electrophoretic mobilities (c3 to c7), which are not quantitatively immunoreactive with AML-1, AML-2, or AML-3 antibodies, suggests that the heterogeneous subcellular partioning of AML factors observed in cellular fractions from calvarial osteoblasts involves distinct AML proteins.

Interestingly, we also observed a weak supershift with the AML-3 antibody in the presence of ROB nuclear matrix protein (Fig. 2C), but there is no concomitant loss of AML complexes in the nuclear matrix fraction. In addition, we have previously shown that nuclear matrix-related AML complexes from ROS 17/2.8 osteosarcoma cells are weakly immuno-reactive with AML-1 antiserum reflected by formation of a minor AML-1 specific supershift [18]. The c5 and c6 subcomplexes formed by ROS 17/2.8 nuclear matrix proteins are also very weakly reactive with AML-3 antiserum (data not shown). However, AML-3 complexes detected with nuclear extracts from ROS 17/2.8 cells (i.e., c1 and c2 subcomplexes) are completely reactive with AML-3 antiserum (Banerjee et al., 1997). Taken together, it appears that the epitopes of AML-1 and AML-3 proteins in some subcellular compartments of osseous cells are not readily accessible to the antibodies used in our studies. Alternatively, the epitopes for antibodies against AML-1 [18] and AML-3 (as well as AML-2) may be absent in several of the AML gel shift subcomplexes detected in our experiments.

Fig. 2. Multiple isoforms of the AML class of tissue-specific transcription factors partition in distinct subcellular compartments. The identity of AML-related isoforms with different electrophoretic mobilities was simultaneously analyzed using gel shift assays with fractions derived from the nuclear matrix (NM) procedure (CSK, RSB, DIG, and NM) and nuclear extract (NE) from differentiated osteoblasts (ROB d22). **A:** DNA competition assays in the presence of 100-fold excess of unlabelled AML (**lane S**) or E2F (**lane N**) consensus oligonucleotides, or in the absence of DNA competitor (**lane C**). The arrowheads designate AML-specific subcomplexes predominantly observed in the CSK (c2 and c3) and RSB (c1 and c4) fractions. Subcomplexes c1 and c2 are observed as a single band in reactions with nuclear extracts (complex AML-I) (see Fig. 1B). Subcomplexes c5 and c6 are nuclear matrix-related, but are detectable at low levels in the DIG fraction. Subcomplexes c5 and c6 are detected as a heterogeneous band ("smear"), designated AML-II, on darker autoradiographic exposures (e.g., see Fig. 1B). **B:** Gel shift immuno-assays using specific rabbit polyconal antisera (α) against AML-1, AML-2, and AML-3, as well as a non-specific antiserum against E2F-1. Subcomplexes are designated as described for A. Immunoreactivity of the c1 and c2 subcomplexes is reflected by the loss of signal in the presence of the AML-3 antibody. Depending on the binding conditions, this loss of signal may occur in conjunction with a visible supershift (see C). **C:** Gel shift immuno-assays as described in B using NE and NM proteins. Presence of the AML-3 antibody results in quantitative absence of the c1 and c2 subcomplexes observed with NE protein with concomitant formation of a supershift complex (αS) immediately above an antibody related non-specific band (αns). A minor AML-3 specific supershift is also observed with NM proteins, but does not result in loss of signal for the c5 and c6 subcomplexes; this may be due to stabilization (or antibody-induced concentration at this specific electrophoretic position) of AML-3 related protein/DNA complexes that remain below the level of detection in the control lane.

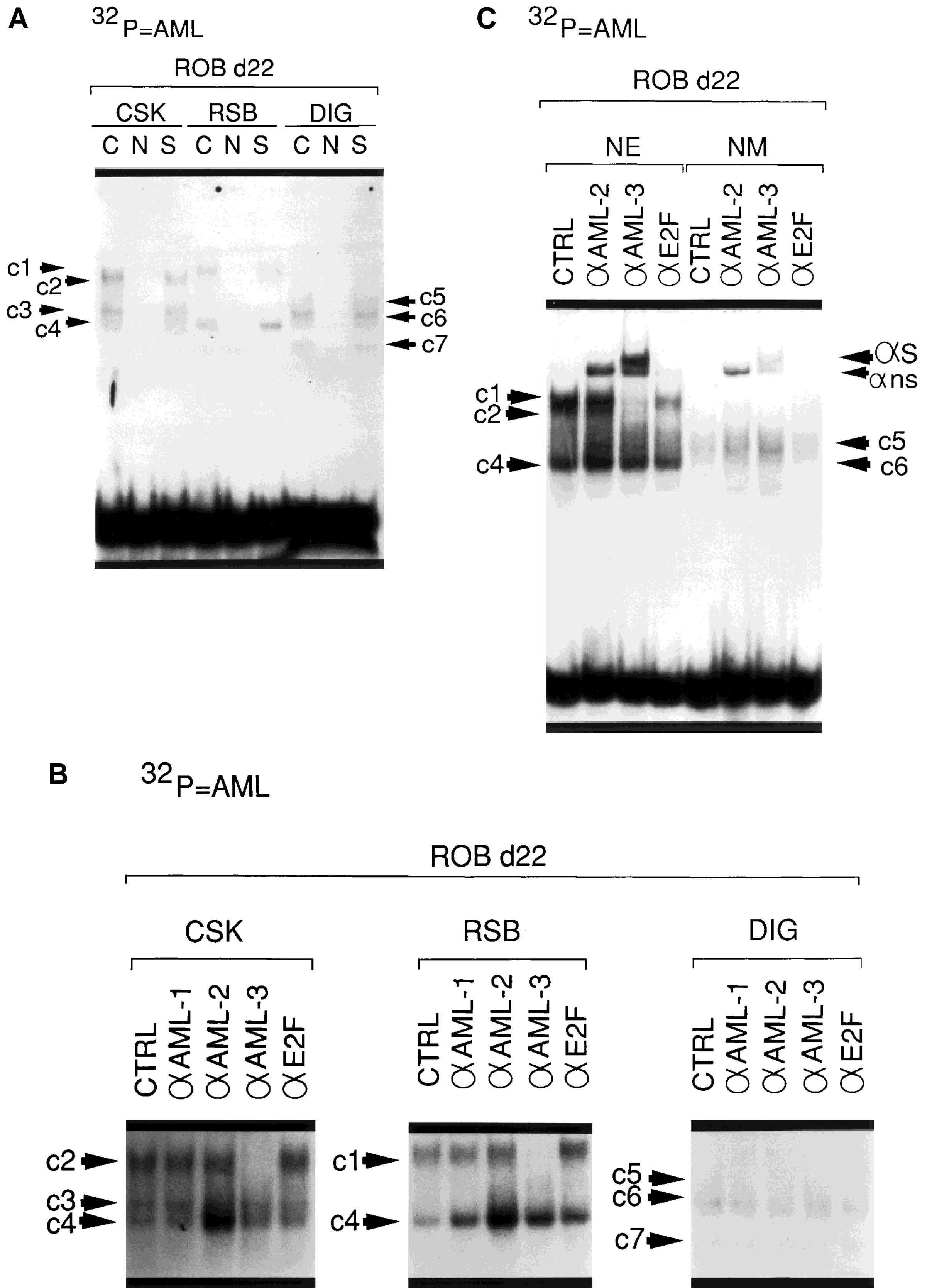

Figure 2.

Developmental Control of Subnuclear Partitioning of AML Proteins in Matrix and Non-Matrix Nuclear Compartments of Osteoblasts

To evaluate whether subnuclear partitioning of transcription factors is modulated during osteoblast differentiation, we analyzed the levels of YY-1, AML, AP-1, and SP-1 in non-matrix nuclear (NE) and nuclear matrix (NM) fractions derived from osteoblasts in the proliferative (day 2) and differentiated (day 22) stages (Fig. 3). The results show that AP-1 binding activity is highest and constitutively present in the non-matrix nuclear compartment, while present at levels below detection in the nuclear matrix at both developmental stages (Fig. 3C). SP-1 activity is also detected only in the NE fraction and the levels of SP-1 in ROB cells are elevated upon differentiation (Fig. 3D). To confirm that this increase in SP-1 binding activity is significant, we analyzed three additional DNA binding activities (e.g., ATF and E2F), which remain at the same level or show a decline during osteoblast differentiation (data not shown).

The results also show that the levels of YY-1 are similar in both the non-matrix nuclear (NE) and nuclear matrix (NM) fraction at either day 2 or day 22 (Fig. 3A). Thus, partitioning of YY-1 in two different subnuclear compartments occurs constitutively during osteoblast differentiation. Strikingly, high levels of AML binding activity are restricted primarily to the differentiated stage of osteoblasts (Fig. 3B). The results show that activity of the AML-I complex (composed of subcomplexes c1 and c2 in Fig. 2) is upregulated in the non-matrix nuclear (NE) compartment, while the AML-II complex (composed of subcomplexes c5 and c6 in Fig. 2) is strongly upregulated in the nuclear matrix compartment during osteoblast differentiation. We conclude that the two major types of AML transcription factor complexes are both selectively upregulated during maturation of the bone cell phenotype. It appears that cells tightly regulate association of distinct AML factors with the nuclear matrix, while YY-1 is capable of partitioning in both subnuclear compartments at the proliferative as well as differentiated stages of osteoblast differentiation.

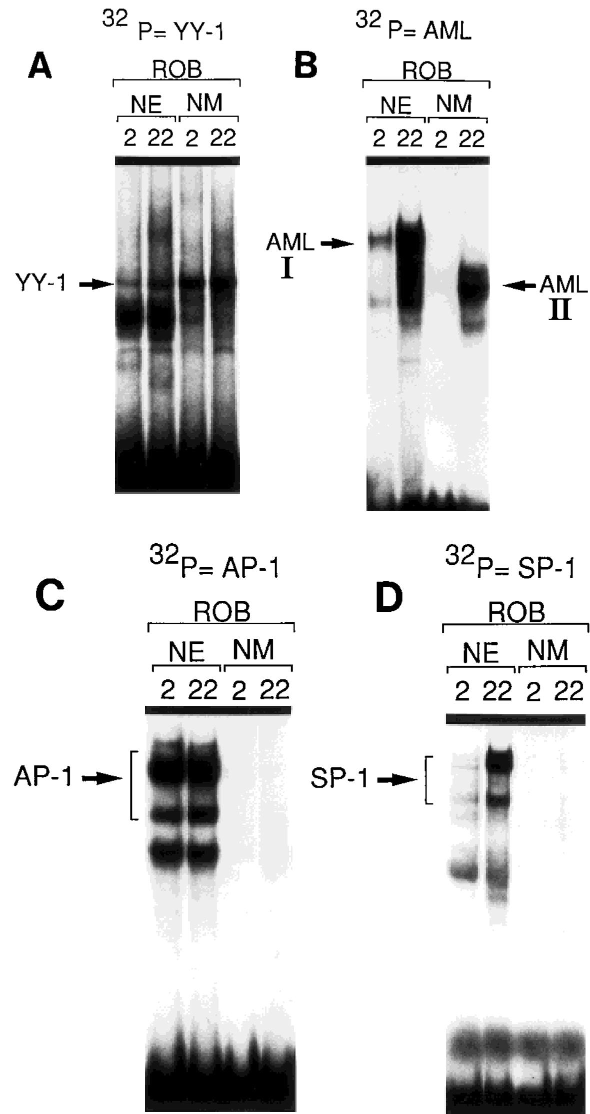

Fig. 3. Subnuclear partitioning of transcription factors during osteoblast differentiation. The levels of YY-1 [A], AML [B], AP-1 [C], and SP-1 [D] binding activities in non-matrix nuclear (NE) and nuclear matrix (NM) fractions derived from osteoblast in the proliferative (day 2) and differentiated (day 22) stages were analyzed by gel shift assays. The arrowheads point at the relevant gel shift complexes as described in Figure 1.

DISCUSSION

In this study we have shown that several distinct transcription factors including NMP-1/YY-1, NMP-2/AML, AP-1, and SP-1 partition differentially between nuclear matrix and non-matrix nuclear compartments during osteoblast differentiation. This finding extends our previous observation that the interaction of transcription factors with the osteoblast nuclear matrix is cell type and/or cell growth related [9,33]. Moreover, using gel shift immuno-assays, we have shown that the bone-related nuclear matrix protein NMP-2 is distinct from AML-3 related complexes, which are present in the non-nuclear matrix fractions (e.g., CSK-, RSB-, and nuclear extract-fractions) of osteo-

blasts [37,41]. We also showed that multiple isoforms of the AML class of tissue-specific transcription factors partition in distinct subcellular compartments. These findings are consistent with extensive heterogeneity in the subcellular location of different AML-related isoforms and AML-derived deletion mutants observed by Western blotting experiments and in situ immunofluorescence analyses [19,42].

Our principal finding is that the association of the NMP-2/AML transcription factor with the nuclear matrix is developmentally upregulated during maturation of the osteoblast phenotype. NMP-2 is immunologically related but not identical to the tissue-specific transcription factor AML-1B [18], which unlike its inactive isoform AML-1, is a potent nuclear matrix associated trans-activator [19]. Three NMP-2 binding sites have been identified in the promoter of the bone-specific osteocalcin gene [33], and at least one site is essential for bone-tissue specific transcription [37,43]. Therefore, the differentiation-dependent association of NMP-2/AML with the nuclear matrix in osteoblasts appears to be functionally involved in the competency for bone-tissue specific transcription. It has recently been shown that AML-3 is the principal gene regulatory factor controlling osteocalcin gene transcription [37,41]. This study reveals that distinct AML-3 proteins and AML-related DNA binding activities are present in osteoblasts and associate with different subcellular compartments.

Our basic premise is that there exists a functional relationship between nuclear architecture and gene expression [8]. We are postulating that the nuclear matrix concentrates gene regulatory factors, and this association of transcription factors with the nuclear matrix contributes to control of gene expression. This model invokes specific protein/nuclear matrix interactions, which can be physiologically modulated in response to intra- and extra-cellular cues. Using osteoblast biology as a paradigm, we have shown previously that tissue-specific transcription factors contain precise nuclear matrix targeting signals [19] and that the composition of the nuclear matrix is modulated during osteoblast differentiation [26] and in response to physiologic mediators of bone-tissue specific gene expression [28,29]. In this study, we have provided evidence that the developmental association of transcription factors with the nuclear matrix in osteoblasts is selective. The nuclear matrix parameters investigated in this study may accomodate modifications in gene expression during bone-tissue development and provide a foundation for further pursuing involvement of the nuclear matrix in gene regulatory mechanisms in osseous cells.

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