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ARTICLES

Sequence-Specific DNA Binding Activities of Nuclear Matrix Proteins of Mammalian Lens Epithelial Cells

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Abstract This study examines matrix and nonmatrix nuclear proteins of the rabbit lens epithelial cells. The nuclear matrix proteins were isolated by modified Penman technique, which requires presence of detergents and nucleases, whereas nonmatrix nuclear proteins were obtained by high salt extraction. The data from these experiments revealed presence of DNA binding activities for SP-1 and OCT-1 proteins in both matrix and non-matrix compartments of rabbit lens epithelial cells. Comparison of the relative abundance of SP-1 and OCT-1 binding activities in nuclear matrix and nonmatrix fractions suggest the distribution between these two compartments is cell type specific and possibly related to the control of cell growth.

Key words: DNA-binding proteins, lens epithelial cell, nuclear matrix, Oct-1, SP-1

The organization and composition of the nuclear matrix (NM) has been attributed to the structural and functional control of gene expression [1,2]. The nuclear matrix has been shown to be composed of peripherally located lamin/pore complexes and internally ubiquitous proteins (matrin) [3,4], heterogenous nuclear RNAs [5,6], and many low abundance proteins [7]. Recent reports have clearly indicated that nuclear matrix proteins are at least in part cell type specific [7,8], tumor related [9–11], and developmentally regulated [12,13].

Many well-established regulators of gene expression have been shown to be associated with the nuclear matrix, including type II DNA topoisomerase [14,15], viral regulatory proteins [16–18], steroid hormone receptor [19,20], and oncogene encoded nuclear proteins [21]. Two types of DNA binding factor interactions with the nuclear matrix have been characterized. The first interacting factor [22] that binds to dA/dT rich DNA sequences is typically found in matrix attachment regions (MARs) [23]. The second type of interactions involve short consensus elements which exhibit sequence-specific recognition for nuclear matrix associated transcription factors. These include, but are not restricted to, NMP-1, which is an ATF-related nuclear matrix protein that binds to the cell cycle regulated histone gene promoter [24] and NMP-2, which is a C/EBP-related nuclear matrix protein that binds to the bone specific osteocalcin gene promoter [19]. Additionally, evidence for involvement of the nuclear matrix in the regulation of gene expression is provided by developmental, growth regulated, and phenotype-related partitioning of transcription factors (e.g., SP-1, OCT-1, and AP-1) between the nuclear matrix and non matrix nuclear fractions [9]. Taken together, these results indicate that many transcription factors are present in the nuclear matrix may contribute to the control of gene regulation via nuclear matrix composition and organization.

In this study we examined the nuclear matrix proteins of rabbit lens epithelial cells for the presence of sequence specific DNA binding proteins that are transcription factors. We observed that two key transcription activities (SP-1, OCT-1) are present in the NM proteins of lens epithelial cells.

MATERIALS AND METHODS

Protein Preparation

The nuclear matrix proteins were obtained by the modified Penman technique [7,19] using confluent rabbit lens epithelial cells grown in

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tissue culture [25]. The matrix proteins were extracted in a series of buffers containing detergent and nucleases to produce the NM-1F scaffold [25]. The NM-1F scaffold was solubilized in disassembly buffer (8M urea, 20 mM MES, 1 mM EGTA, 12 mM PMSF, 0.1 mM $MgCl_2$, 1% βME at pH 6.6) and then dialyzed against assembly buffer (0.15 M KCl, 2.5 mM imidazole hydrochloride, 5 mM $MgCl_2$, 2 mM DTT, 0.125 mM EGTA, 0.2 mM PMSF at pH 7.1) for 12 h. The dialysate was centrifuged at 100,000*g* for 95 min to separate soluble NM proteins from the pellets of internal nuclear membrane. Nonmatrix nuclear factors (NE) were obtained by 0.42 M KCl extraction of isolated nuclei by Dingnam technique [26]. The protein amounts were quantitated by Bradford analysis [19].

Gel Shift Analysis

SP-1 and OCT-1 oligonucletides were used as probes in gel shift assays and are presented in Figure 1 (see also Table I). Probes were prepared as described previously [27], by labelling with p32 using T-4 polynucleotide kinase. Binding reactions were performed in a final volume of 20 μl. Each mixture contained 200 ng poly (dI-dC) poly (dI-dC), 15% glycerol, 75 mM KCl, 16 mM Hepes, 0.15 mM EDTA, and 10 fmol of probe DNA and 1.0 μg of protein from either matrix or nonmatrix preparation [9]. Electrophoretic separations were performed in 5% (80:1) polyacrylamide in 1× TGE gels [28], followed by autoradiography.

Competition analysis was performed by addition to the binding reaction of a 100-fold excess of either specific competitor DNA or an unrelated oligonucleotide of similar size.

Quantitation of protein/DNA interactions was performed by image analysis (GDS 2000, Gel documentation system, San Gabriel, CA) of short autoradiographic exposures of the gel shift assay PAGE profile.

Tissue Culture of Mammalian Lens Epithelial Cells

The lens epithelial cells were obtained from 6-month-old male rabbits. These cells were grown in T-150 Falcon flasks. The cell monolayers were grown at 37°C in Dulbecco's medium (DMEM) supplemented with 10% calf serum and 10% rabbit serum in a humidified atmosphere of 5% CO_2 and 95%/air. The confluent lens epithelial cells after ten passages were washed with phosphate buffered saline and removed from the surface of the culture plate by rubber policeman.

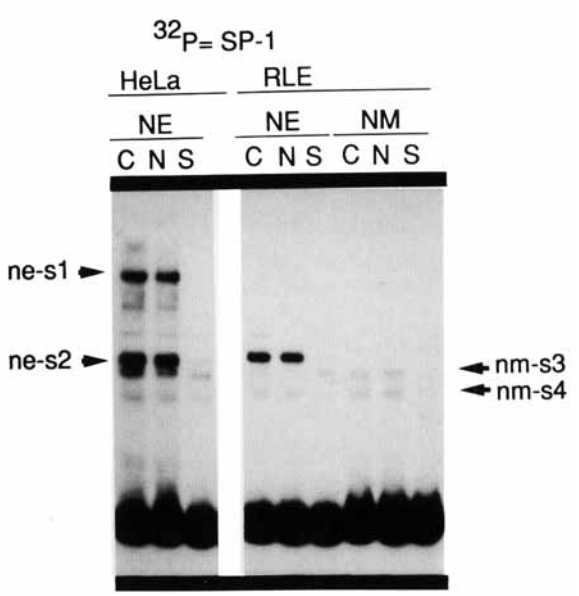

Fig. 1. Presence of SP1-related binding activities in nuclear matrix and nonmatrix nuclear preparations. Gel shift analysis was performed using a consensus SP-1 oligonucleotide (10 fmol) in the presence of 200 ng poly I/C nonspecific competitor DNA substrate. Binding reactions were performed with 1 μg of protein derived from the nonmatrix (NE) and nuclear matrix (NM) compartments of rabbit lens epithelial cells (RLE) (**right panel**) or with nonmatrix nuclear proteins from human HeLa cervical carcinoma cells (**left panel**) as a control for SP-1 detection. Sequence specificity of the protein/DNA interactions was confirmed by competition analysis using 100-fold excess of unlabelled competitor DNAs. S, SP-1 oligonucleotide; N, nonspecific CCAAT-oligonucleotide; C, binding reaction in the absence of competitor oligonucleotide. Arrowheads: SP-1 related DNA binding activities observed with nonmatrix nuclear proteins (NF-S1 and NE-S2) and nuclear matrix proteins (NM-S3 and NM-S4).

TABLE I. Summary of Oligonucleotides Used for Detection of Transcription Factors*

SP-1 5′CGGATGGGCGGGGCCGG
GGATGGGCGGGGCCGG-3′
OCT-1 5′AACTCTTCACCTTATTTGCA
TAAGCGATCTACTGCTC-3′

*Specific consensus binding sequences for each factor are underlined. The high affinity SP-1 binding sites are based on consensus sequences [Mitchell and Tijan, 1989]. OCT-1 (histone H2B) [Fletcher et al., 1987] binding sites were synthesized according to the natural sequences found in the promoters of the indicated genes.

RESULTS

These experiments were performed to assay for the presence of known transcription factors like SP-1 and OCT-1 in the nuclear matrix pro-

teins. As described in Materials and Methods, nuclear matrix proteins were isolated by solubilization of nuclear matrixes that were obtained by sequential detergent extraction and extensive DNase and RNase digestion [7]. Nonmatrix nuclear proteins were extracted by 0.42 M KCl and mainly contain numerous low abundance nonhistone chromatin proteins, including many gene specific transcription factors [9].

DNA Binding Activities of SP-1 Are Present in Both the Nuclear Matrix and Nonmatrix Nuclear Proteins

SP-1 is a ubiquitously present transcription factor which supports basal transcriptional activities of a broad spectrum of eukaryotic gene promoters [29]. We therefore assayed SP-1 related binding activity in the NM and NE compartments of rabbit lens epithelial cells by determining sequence specific protein/DNA interaction of NM and NE fractions. As indicated in Figure 1, SP-1 related binding activities were observed in both the NM and NE fractions. However, differences in the mobilities of the SP-1 related protein/DNA complexes are clearly evident. NE-S2 activity is seen solely in NE, while NM-S3 is predominantly evident in the NM proteins. NM-S4 appears to partition between the NM and NE compartments. The specificity of these protein/DNA interactions is reflected by the absence of competition with unrelated DNA oligonucleotides.

Association of the Cell Growth Regulated Transcription Factor OCT-1 With NM and NE Protein

OCT-1 is a POU/homeodomain transactivation factor which recognizes the octameric ATTTGCAT motif located in the flanking regions of many genes [30].

It is not surprising that two principal protein-DNA complexes were observed (designated 01 and 02), because OCT-1 belongs to a multigene family of transcription factors that exhibit expression in association with both development and tissue phenotype maintenance. While OCT-1 appears to be preferentially active in proliferating cells, maintenance of functional integrity of tissues in culture necessitates division and remodeling; thus, the presence of OCT-1 is not surprising.

In HeLa cell nuclear extracts two principal OCT-1 protein/DNA complexes were observed (NE-01, NE-02) (Fig. 2). In both NM and NE proteins from rabbit lens epithelial cells NM-02 is the primary OCT-1 related complex which exhibits sequence specific DNA binding activity. The apparently similar representation of NM-02 in both NM and NE compartments is similar to the observed distribution of OCT-1 in NM and NE compartment of Hela S3, ROS 17/28 MG-65, UMR-106 cells [9].

DISCUSSION

The mammalian lens epithelial cells present an ideal model system to study cell proliferation and differentiation. These cells in vivo are predominantly maintained in mitotic quiescence and express mainly tissue specific proteins (alpha, beta, and gamma crystallins). However, quiescent epithelial cells can be triggered to proliferate, thus stimulating nuclear mechanisms that control activation of cell cycle related genes. After a few passages in tissue culture lens epithelial cells loose their ability to synthesize tissue specific crystallins, but retain diploid char-

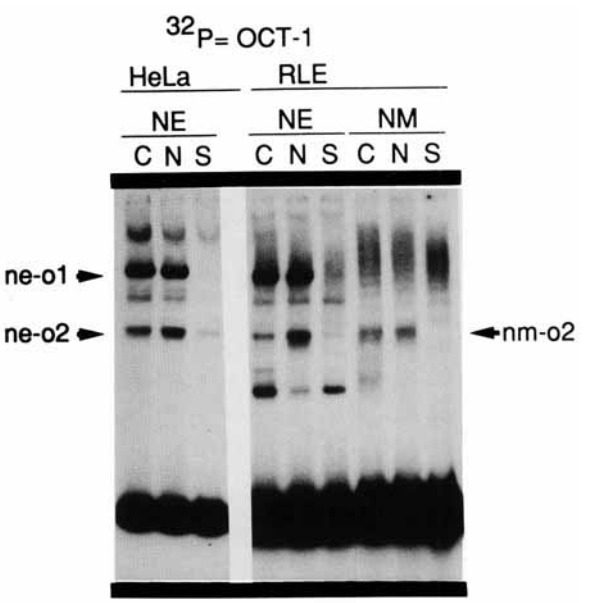

Fig. 2. Presence of OCT-1 related binding activities in nuclear matrix and nonmatrix nuclear preparations. Gel shift assays were performed as described in the legend to Figure 1 using a consensus OCT-1 oligonucleotide. Proteins present in the binding reaction were derived from nonmatrix (NE) and nuclear matrix (NM) compartments of rabbit lens epithelial cells (RLE) (**right panel**) or with nonmatrix nuclear proteins from HeLa cells as control for detection of OCT-1 activity (**left panel**). Sequence specificity of the indicated protein/DNA complexes from nonmatrix (NE-01 and NE-02) and matrix (NM-02) fractions was established as described in Figure 1. S and N, respectively, unlabelled specific OCT-1 and nonspecific CCAAT oligonucleotide; C, binding reactions in the absence of competitor oligonucleotides.

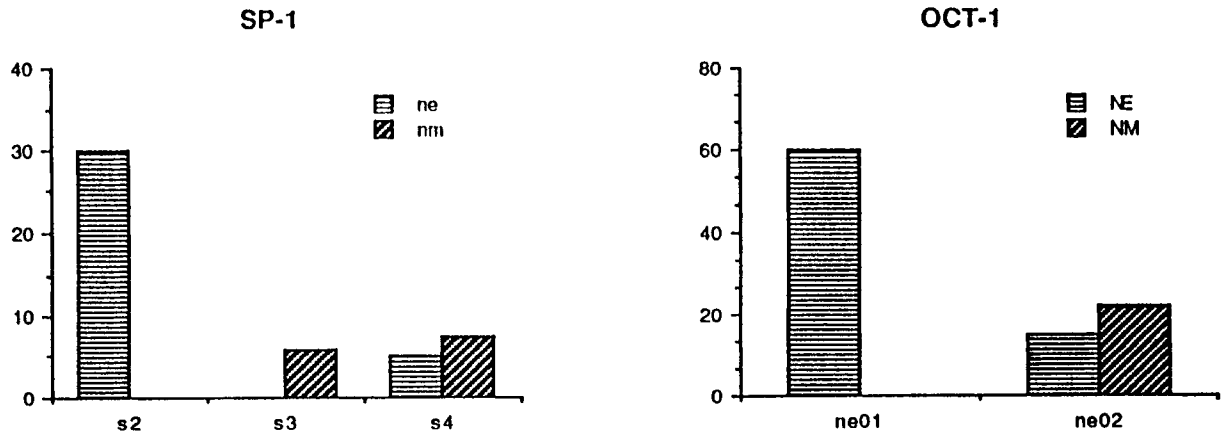

Fig. 3. Cell type dependent differences in the specific activity of transcription factors in the matrix and nonmatrix nuclear preparation. Quantitation of binding factor activities was obtained from analyzing the relative intensity of gel shift complexes' (indicated vertically and expressed as an arbitrary unit) representative autoradiograms. This graph compares the amount of the binding activities monitored with the SP-1 (**left**) and OCT-1 (**right**) probes using identical quantities of proteins (1.0 μg) of matrix and nonmatrix nuclear fractions isolated from rabbit lens epithelial cells.

acter. It can be postulated that during tissue culture, lens epithelial cells lose the structural integrity which prevents expression of tissue specific proteins. In tissue culture, lens epithelial cells synthesize crystallins for the first few generations and then stop expression of crystallins. If, as proposed, NM proteins are involved in the control of tissue specific gene expression, lens epithelial cells provide an excellent model system for the further elucidation of regulatory mechanisms. A functional relationship between NM proteins and transcriptional control is further suggested by the progressive modification in the complement of these proteins in parallel with changes in gene expression during bone cell differentiation [24].

In this investigation we observed the presence of DNA binding activities for SP-1 and OCT-1 proteins in both NM and NE compartments of cultured rabbit lens epithelial cells. The apparent association of these factors with the NM occurs in several cell types [9] (Fig. 3). Our data also support a postulated role of the NM in the regulation of gene expression, which involves the selective localization of a subset of transcription factors resulting in the regional concentration of low abundance positive or negative transactiving proteins [31]. The NM may additionally serve as a reservoir by sequestering regulatory proteins in a dormant state [9].

It has been proposed that the NM is structurally and functionally related to transcriptional regulation by providing a link between matrix attachment regions (MARs) and the transcriptional activity of a gene [24,32,33]. Both tenacious and transient attachment domains have been postulated. We have also shown that there is differential presence of SP-1 in the NM and NE compartments. This ubiquitous protein may contribute to regulatory activity by shuttling between two subnuclear compartments. The clustering and distinct permutations of binding sites in many gene promoters raise the possibility that some aspects of gene nuclear matrix attachment could be intrinsic properties of transcription factors [9]. These concepts will be further defined experimentally using the lens epithelial cell model system.

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