Journal of Cellular Biochemistry 61:467–477 (1996)

Association of Transcription Factors With the Nuclear Matrix

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Abstract The nuclear matrix is the framework scaffolding of the nucleus and has been demonstrated to be an important component in a number of nuclear processes including transcription, replication, and RNA splicing and transport. In the interphase nucleus, DNA is specifically organized in a three-dimensional fashion. An example of this fact is that actively transcribed genes have been demonstrated to associate with the nuclear matrix. In this study, nuclear matrix proteins from various rat tissues, including two androgen-regulated tissues, the seminal vesicle and ventral prostate, were examined to determine if they contained proteins that associate with consensus binding sequences for several proteins involved in the regulation of transcription. Specific interactions were identified between proteins of the nuclear matrix and these transcriptional activator binding sequences. In addition, the sizes of the complexes binding to the DNA sequences appeared to vary in some of the tissues. These data support the concept that the nuclear matrix may serve as a support structure to bring together specific DNA sequences with factors involved in the regulation of gene expression.

Key words: nucleus, nuclear scaffold, nuclear skeleton, gene regulation, DNA

In the interphase nucleus, the 30 nm chromatin filaments form approximately 50,000 DNA loop domains, each of approximately 60 kbp, that are attached at their bases to the inner portions of the nuclear matrix [Pardoll et al., 1980]. The nuclear matrix is the framework scaffolding of the nucleus and consists of the peripheral lamins and pore complexes, an internal ribonucleic protein network, and residual nucleoli [Berezney and Coffey, 1974]. The nuclear matrix may be isolated by salt extraction of the nucleus, consists of approximately 10% of the nuclear proteins, and is virtually devoid of lipids, DNA, and histones [Fey et al., 1991]. A majority of the nuclear matrix proteins identified are common to all cell types and physiologic states, while a specific group of nuclear matrix proteins are tissue specific and others are altered with the state of the cell, such as transformation [reviewed in Getzenberg, 1994].

Received September 15, 1995; accepted December 5, 1995.

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The nuclear matrix forms a three-dimensional organizing site for many nuclear functions. DNA replication has been shown to occur on the nuclear matrix, at fixed sites at the base of the loops in a complex termed the replisome. The replicon units, equivalent to the 60 kpb DNA loop domains [Pardoll et al., 1980], are synthesized in a precise order and temporal sequence. This replicon has now been shown to be localized on the nuclear matrix. The nuclear matrix also plays a central role in organizing RNA processing and has been shown to be the site of attachment for products from RNA cleavage and for RNA processing intermediates [Carter and Lawrence, 1991; Xing et al., 1993]. Spliceosome complexes involved in the regulation of RNA splicing have been localized to the nuclear matrix. Newly synthesized heteronuclear RNA and small nuclear RNA are enriched on the nuclear matrix. RNA [He et al., 1990; Spector, 1990] and ribonucleoprotein particles and fibers [Harris and Smith, 1988; Nickerson et al., 1989; Van Eekelen and Van Venrooij, 1981] may themselves have an important role in the structure of the nuclear matrix.

The structural components of the nucleus are known to have a central role in the specific

topological organization of DNA. DNA in the nucleus is not randomly organized and although only approximately 10% of the DNA actually encodes genes, only specific genes are positioned in a manner that permits the expression of both housekeeping and cell type–specific genes. The average mammalian somatic cell nucleus contains a linear equivalent of 2 m of DNA packed by a 200,000-fold, linear condensation into a nucleus 10 μ in diameter. The DNA has many forms of higher order structure which are organized in a particular pattern which results in the expression of only appropriate tissue-specific genes. With the use of in situ hybridization [McNeil et al., 1991], there is now direct evidence for specific three-dimensional organization of the DNA within the nucleus [Carter and Lawrence, 1991; Haaf and Schmid, 1991]. Manuelidis and coworkers demonstrated the specific and reproducible compartmentalization of unique chromosomal domains within the nuclei of human central nervous system cell lines and established that functionally distinct cell types have specific patterns of three-dimensional organization of the interphase chromosomes [Manuelidis and Borden, 1988]. The use of confocal microscopy has further enabled the visualization of multiple probes simultaneously and has confirmed the dynamic and specific cellular localization of specific genes within the nucleus [Lichter et al., 1988; Lichter and Ward, 1990]. In summary, many studies have now demonstrated the specific three-dimensional organization of DNA within the nucleus. We hypothesize that differences in this organization can occur with the same genomic sequence and that they are dictated in part by DNA interactions with a tissue-specific nuclear matrix [Getzenberg, 1994]. According to this hypothesis, nuclear structure may determine both the topological organization of DNA and the functional effects which result from this organization.

The nuclear matrix has also been demonstrated to be the site of mRNA transcription. Active genes have been found to be associated with the nuclear matrix only in cell types in which they are expressed [Getzenberg, 1994]. Genes that are not expressed in these cell types are not found to be associated with the nuclear matrix. In addition, transcription factors including the myc protein, the large T antigen of the SV40 virus, and E1A from adenovirus have all been found to be associated with the nuclear matrix [Covey et al., 1984; Eisenman et al., 1985; Sarnow et al., 1982; Staufenbiel and Deppert, 1983]. Recent evidence has further demonstrated that several transcription factors are localized or sequestered on the nuclear matrix and that this localization may play an important role in the regulation of gene expression [Getzenberg and Coffey, 1991; van Wijnen et al., 1993]. Stein and his colleagues have examined the regulation of the osteocalcin gene and have identified multiple protein–DNA interactions in the promoter region, involving two different nuclear matrix proteins. These proteins interact with regions of the gene in proximity to the vitamin D–responsive sequences and are related or identical to known transcription factors [Bidwell et al., 1993].

The nuclear matrix has been shown to play a central role in the action of certain hormones, particularly steroid hormones. Extensive work has identified high affinity binding of steroid receptors to the nuclear matrix in many estrogen- and androgen-responsive tissues [Barrack, 1987], including the ventral prostate [Barrack and Coffey, 1980] and seminal vesicle [Epperly et al., 1984]. Using the techniques of labeling in vivo, exchange reactions in vitro, and cell-free reconstitutions, steroid receptors have been found to be present in the nuclear matrix of steroid-responsive tissues. The binding of steroid receptors to the nuclear matrices of individual tissues is both steroid and tissue specific, and requires the presence of an activated steroid receptor complex with bound steroid. For example, androgens were found to bind to the nuclear matrices of androgen-responsive tissues and estrogens to the nuclear matrices of estrogen-responsive tissues [Barrack and Coffey, 1980]. Tissues that do not demonstrate hormone responsiveness are not found to have hormone receptors on their nuclear matrices. In addition, the level of steroid receptor binding in these nuclear matrices was demonstrated to vary with the hormonal state of the animal. Receptors were not found bound to the nuclear matrix in the hormone withdrawn state; with subsequent administration of specific hormones the receptor complexes were then again shown to bind the nuclear matrix.

Steroid receptors interact with the nuclear matrix through acceptor sites which are present on the nuclear matrix and are involved in the saturable, high affinity, specific binding described above. The acceptor protein for the progesterone receptor, termed RBF-1, has been well

characterized [Schuchard et al., 1991a,b] as a nuclear matrix protein with a high affinity for the progesterone receptor. It is proposed that it is through these acceptor proteins that steroid receptors interact with the nuclear matrix and the DNA, and therefore may position themselves in the appropriate location for the activation and/or repression of gene expression [Landers and Spelsberg, 1992].

These results support the concept that transcription factors are localized or sequestered on the nuclear matrix, and that such localization on the nuclear matrix may play an important role in DNA binding and the specific organization and regulation of gene expression. This study examines the interaction of several transcriptional activator DNA binding sequences with the nuclear matrix proteins of rat ventral prostate and seminal vesicle, demonstrating that these transcriptional activators are associated with the nuclear matrix.

MATERIALS AND METHODS

Animal Procedures

This investigation conforms with the Guidelines for Care and Use of Experimental Animals. Sprague–Dawley male rats were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). These rats were fed and housed under identical conditions, with each rat weighing between 325 and 349 g and aged 78–91 days old.

Nuclear Matrix

Nuclear matrix proteins were isolated from fresh rat ventral prostates and seminal vesicles according to an adaptation of the methodology of Fey and Penman [Fey et al., 1991; Getzenberg and Coffey, 1990]. Briefly, the tissues were minced into small pieces and then homogenized on ice with 0.5% Triton X-100 to release the lipids and soluble proteins in a buffered solution containing 2 mM vanadyl ribonucleoside, an RNase inhibitor. The extract is filtered through a nylon mesh to remove connective tissue and large debris. Salt extraction is performed with 0.25 M ammonium sulfate with 2 mM vanadyl ribonucleoside to release the soluble cytoskeletal elements. DNase I and RNase I treatment at 25°C is used to remove soluble chromatin and RNA. The remaining sample containing intermediate filaments and nuclear matrix proteins is then disassembled with 8 mM urea, and the insoluble components pelleted. Urea is dialyzed out and the intermediate filaments reassemble. The remaining soluble nuclear matrix proteins are then precipitated with ethanol. All solutions contain freshly prepared 1 mM phenylmethylsulfonyl fluoride to inhibit serine proteases. The protein composition is determined by utilizing Commassie Plus Protein Assay (Pierce, Rockford, IL), with bovine serum albumin as standard.

Preparation of DNA Probes

Double-stranded oligonucleotides, purchased from Promega (Madison, WI), represent known consensus sequences of characterized binding sites of transcriptional activators. These oligonucleotides were end labeled with gamma ^{32}P-ATP (Dupont/NEN, Wilmington, DE) and T4 polynucleotide kinase (Promega, Madison, WI). The samples were incubated at 37°C and the reaction stopped by adding 0.5 M EDTA. The unincorporated gamma ^{32}P-ATP was removed using a G-50 Sephadex Column (Boehringer Mannheim Corp., Indianapolis, IN), and the probes were stored at −20°C until utilized. The following probes were used in these experiments: GRE (5′-TCG ACT GTA CAG GAT GTT CTA GCT ACT 3′); CREB (5′-AGA GAT TGC CTG ACG TCA GAG AGC TAG 3′); TFIID (5′-GCA GAG CAT ATA AGG TGA TGA GGT AGG A-3′); AP-1 (5′-CGC TTG ATG AGT CAG CCG GAA-3′).

Gel Retardation Analysis

One-dimensional gel electrophoresis was carried out utilizing the PROTEAN II Xi Cell system (Bio-Rad, Melville, NY) using low ionic strength PAGE. Samples were prepared with 2 μl of prepared 5× buffer (Promega, Madison, WI), 10 μg of appropriate nuclear matrix protein, and, when appropriate, 2 μl of competitor oligonucleotide, the remaining volume consisting of water. The 5× gel binding buffer consisted of 10% glycerol, 5 mM $MgCl_2$, 2.5 mM EDTA, 2.5 mM dithiothreitol (DTT), 250 mM NaCl, 50 mM Tris-HCL, pH 7.5, and 0.25 mg/ml poly (dI-dC)-poly (dI-dC). The reaction mixture was incubated at room temperature for 5 min, then 4 μl of ^{32}P-labeled DNA was added and incubated in a 30°C waterbath for 20 min. To stop the reaction, 1 μl of 10× gel loading buffer, consisting of 250 mM Tris-HCl, pH 7.5, 0.2% xylene cyanol, and 40% glycerol, was added. This also aids in application of the samples to the gel and tracking the progress of the samples

in the gel. HeLa cell nuclear extract (Promega, Madison, WI) was used as a positive control. The reaction mixtures were then run on a 4% acrylamide gel (80:1 acrylamide to bisacrylamide) on the Protean II Xi vertical slab gel system (Bio-Rad, Melville, NY) using a 16 × 20 cm gel length format. The gel mix consisted of 270 μl of 1 M Tris-HCL, pH 7.9, 80 μl of 0.5 M EDTA, pH 8.0, 132 μl of 1 M sodium acetate, 5.33 ml of 30% acrylamide, 1 ml of 2% bisacrylamide, 2 ml of 50% glycerol, and 31 ml of deionized water [Chodosh, 1988]. Electrophoresis buffer consisted of 26.9 ml of 1 M Tris-HCL, pH 7.9, 13.2 ml of 1 M sodium acetate, pH 7.9, 8.0 ml of 0.5 M EDTA, pH 8.0, and water up to a volume of 4 l [Chodosh, 1988]. A pump system was used to constantly recirculate the buffer, and the inner core of the gel apparatus was cooled to 4°C using a recirculating waterbath. The gels were prefocused at 100 V for 90 min prior to the addition of the samples. After the addition of the samples, the gels were run at 150 V, constant, until the dye front approached the lower ⅓ of the gel. The gels were removed from the glass plates and dried using the Hoefer Easy Breeze Gel Dryer (Hoefer, San Francisco, CA). Dried gels were then placed into X-ray film cassettes (Kodak, Rochester, NY) and exposed to film at −70°C.

RESULTS

In order to examine the association of consensus binding sequences of transcriptional activators with the nuclear matrix, gel retardation analysis was performed utilizing consensus binding sequences. The consensus sequences were labeled and incubated with nuclear matrix proteins to determine the presence of binding activity. The interaction of the consensus binding sequence for TFIID, a general transcription factor that exhibits specific DNA binding to the TATA box, with nuclear matrix proteins from numerous rat tissues was examined. TFIID is a term that describes the transcriptionally active holocomplex which contains the TATA box binding protein (TBP). The conditions utilized to prepare the nuclear matrix proteins utilized in these studies are sufficient to separate TBP from its cofactors; therefore, it is possible that we are detecting complex formation with TBP. However, the component(s) of TFIID that we are detecting in these studies is still under investigation. Figure 1 demonstrates that this consensus binding sequence associates with the nuclear matrix of all of the tissues tested. Lane 1 represents the unbound labeled TFIID sequence and lane 7 the positive control HeLa cell nuclear extract. In addition, there appear to be some differences in the size of the complexes formed by these nuclear matrix proteins with this labeled sequence. TBP alone would be expected to

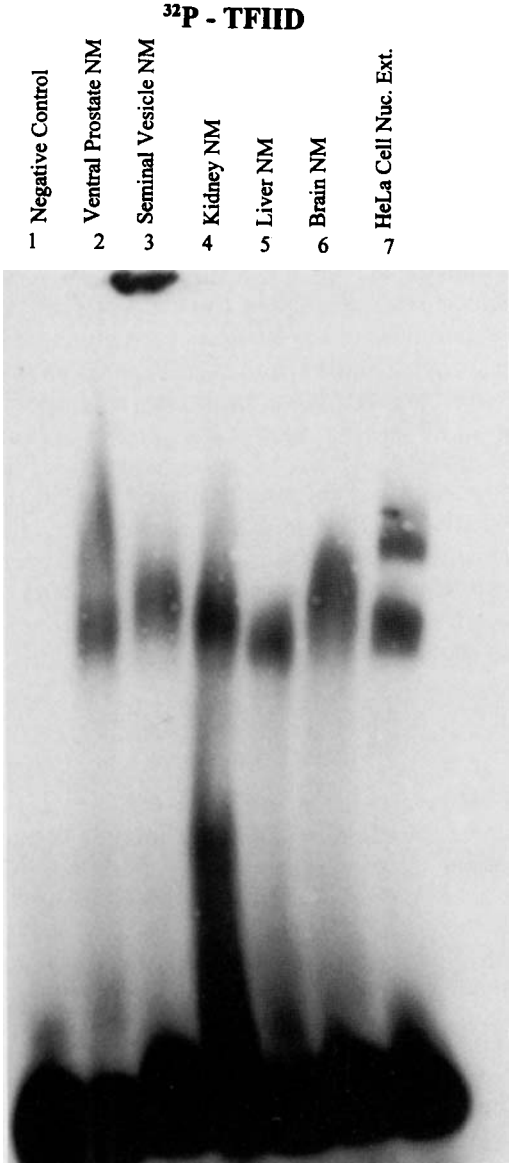

Fig. 1. Presence of TFIID binding activity in the nuclear matrix proteins of numerous rat tissues. Gel retardation analysis of labeled TFIID consensus sequence. *Lane 1* is the labeled DNA sequence without the addition of protein (negative control). Ten micrograms of nuclear matrix protein was incubated with the ^{32}P-labeled consensus sequence of the general transcription factor, TFIID. Nuclear matrix proteins were isolated from rat ventral prostate *(lane 2)*, seminal vesicle *(lane 3)*, kidney *(lane 4)*, liver *(lane 5)*, and brain *(lane 6)*. This figure demonstrates the presence of binding activities in all of the tissues and differences in the complex sizes among some of the tissues examined. *Lane 7* is a positive control for the incubation and gel retardation procedures, and consists of added HeLa cell nuclear extract (Promega, Madison, WI).

form a complex that would run near the free probe under the conditions utilized in these studies. Demonstration of a larger complex lends further credence to the possibility that we are detecting complexes of proteins associated with the nuclear matrix. To determine if this interaction was specific, further analysis was conducted with the nuclear matrix from the rat seminal vesicle, an androgen-regulated male sex accessory tissue (Fig. 2). Lane 1 is the unbound TFIID sequence. Lane 2 demonstrates the retardation of the labeled consensus sequence which is then competed off in lane 3, with excess TFIID. This interaction is specific, as demonstrated by lane 4, which includes the addition of excess quantities of the consensus binding sequence for the unrelated transcription factor, AP-2. As shown in this figure, seminal vesicle nuclear matrix proteins exhibit a specific association with the consensus binding sequence for TFIID.

In order to determine if these interactions were also common to other transcription factor binding sequences, similar experiments were conducted with the glucocorticoid response element, GRE, that confers inducibility to glucocorticoids and has a zinc finger binding domain. The association of the consensus GRE with nuclear matrix proteins prepared from the same rat tissues as described above was then examined (Fig. 3). As described above for TFIID, the GRE consensus sequence associates with all of the nuclear matrix proteins tested, again demonstrating differences in complex sizes among several of the tissues. To determine if this interaction was specific, experiments were again focused on the seminal vesicle nuclear matrix. Figure 4 demonstrates that the interaction of the seminal vesicle nuclear matrix with this GRE consensus sequence is specific.

Examination of the tissue differences in complex formation was performed, comparing the interactions of the rat seminal vesicle nuclear matrix to those of the rat ventral prostate nuclear matrix. Both tissues are androgen responsive and have regulated secretion of proteins into the ejaculate. As evident from Figure 5, when the interactions of the nuclear matrix proteins of the rat seminal vesicle and ventral prostate with the binding sequence for AP-1 were compared, differences in the number of retardations in mobility existed. There appear to be specific multiple bands resulting from the interaction with the seminal vesicle (lane 2) that are not present with the ventral prostate nuclear matrix (lane 7).

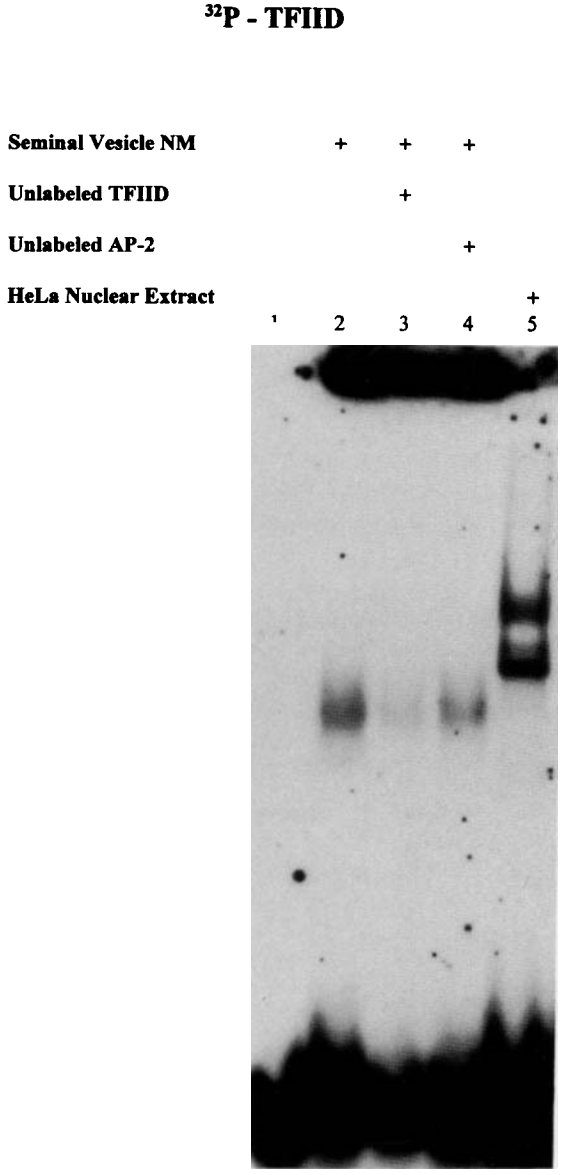

Fig. 2. Presence of TFIID binding activity in the nuclear matrix of rat seminal vesicle. Gel retardation analysis of labeled TFIID consensus sequence. *Lane 1* is the labeled DNA sequence without the addition of protein (negative control). Ten micrograms of nuclear matrix protein was incubated with the ^{32}P-labeled consensus sequence of the general transcription factor, TFIID *(lane 2)*. Specificity of binding was established by competition of the binding with the addition of 100-fold molar excess of cold competitor sequence (TFIID; *lane 3*) and 100-fold molar excess of an unrelated unlabeled consensus binding sequence (AP-2; *lane 4*). *Lane 5* is a positive control for the incubation and gel retardation procedures, and consists of HeLa cell nuclear extract (Promega, Madison, WI).

Similar studies were performed with the consensus binding sequence for CREB, which confers responsiveness to cAMP, contains a leucine zipper motif for dimerization, and possesses an associated basic domain homologous to c-jun

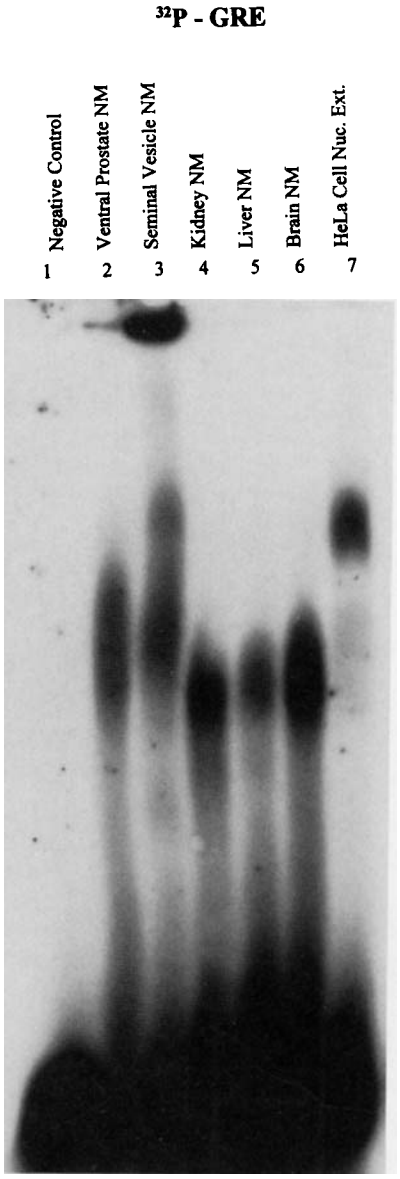

Fig. 3. Presence of GRE binding activity in the nuclear matrix proteins of numerous rat tissues. Gel retardation analysis of labeled GRE consensus sequence. *Lane 1* is the labeled DNA sequence without the addition of protein (negative control). Ten micrograms of nuclear matrix protein was incubated with the ^{32}P-labeled consensus binding sequence of the glucocorticoid receptor. Nuclear matrix proteins were isolated from rat ventral prostate *(lane 2),* seminal vesicle *(lane 3),* kidney *(lane 4),* liver *(lane 5),* and brain *(lane 6).* This figure demonstrates the presence of binding activities in all of the tissues and differences in the complex sizes among some of the tissues examined. *Lane 7* is a positive control for the incubation and gel retardation procedures, and consists of added HeLa cell nuclear extract (Promega, Madison, WI).

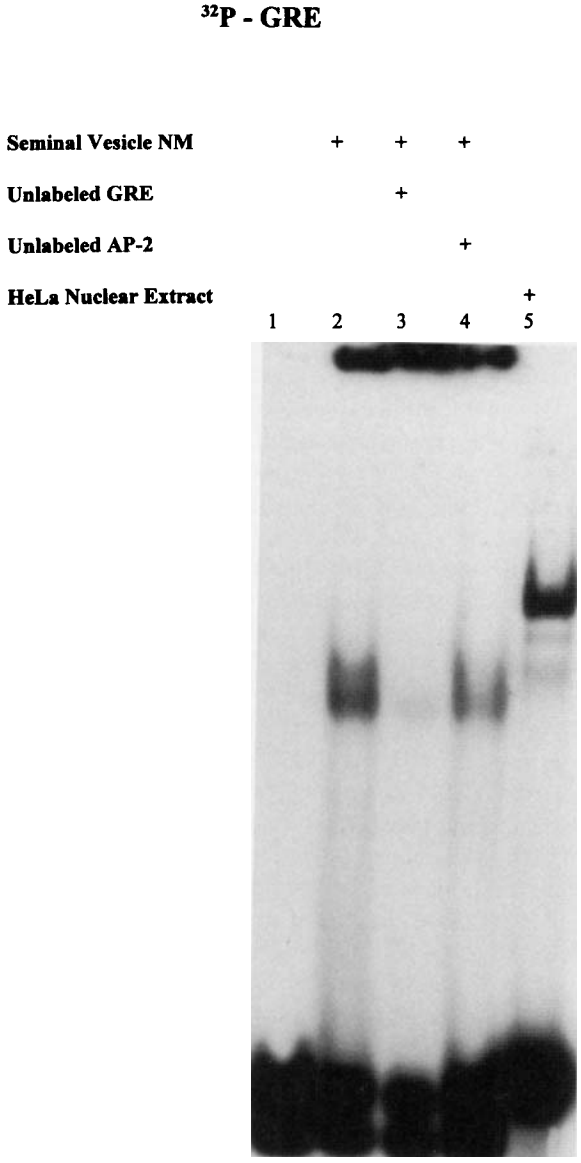

Fig. 4. Presence of GRE binding activity in the nuclear matrix of rat seminal vesicle. Gel retardation analysis of a labeled GRE consensus sequence. *Lane 1* is the labeled DNA sequence without the addition of protein (negative control). Ten micrograms of nuclear matrix protein was incubated with the ^{32}P-labeled consensus binding sequence of the glucocorticoid receptor *(lane 2).* Specificity of binding was established by competition of the binding with the addition of 100-fold molar excess of cold competitor sequence (GRE; *lane 3*) and 100-fold molar excess of an unrelated unlabeled consensus binding sequence (AP-2; *lane 4*). *Lane 5* is a positive control for the incubation and gel retardation procedures, and consists of HeLa cell nuclear extract (Promega, Madison, WI).

DNA binding domains. The CREB sequence associates with both ventral prostate and seminal vesicle but the association is not identical between the two (Fig. 6). The band resulting from incubation with the seminal vesicle nuclear matrix is significantly larger in size then the interaction between the labeled DNA sequence and the rat ventral prostate nuclear matrix. As will be presented in the Discussion, this lends further credence to the concept that at least some of the possible interactions between the nuclear

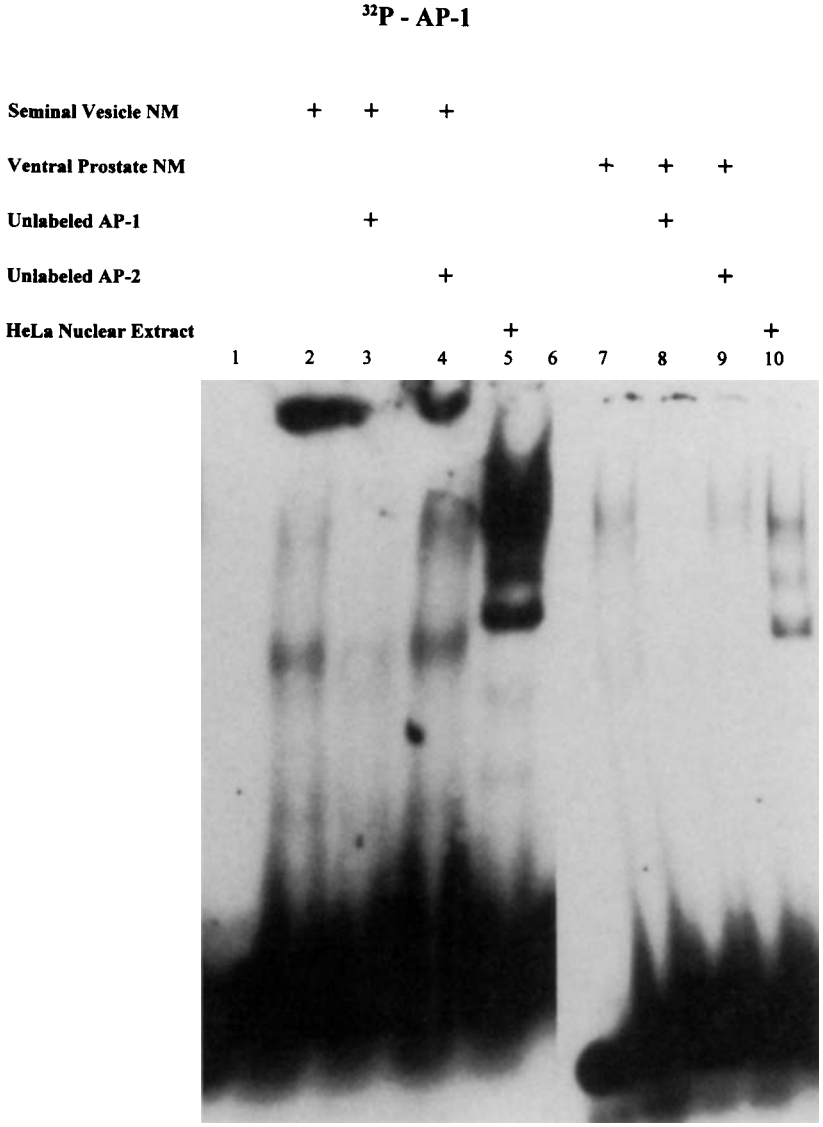

Fig. 5. Presence of AP-1 binding activity in the nuclear matrix of rat seminal vesicle and ventral prostate. Gel retardation analysis of labeled AP-1 consensus sequence. *Lanes 1* and *7* are the labeled DNA sequence without the addition of protein (negative control). Ten micrograms of seminal vesicle and ventral prostate nuclear matrix protein (*lanes 2* and *8*, respectively) were incubated with the ^{32}P-labeled consensus sequence of the transcription factor, AP-1. Specificity of binding was established by competition of the binding with the addition of 100-fold molar excess of cold competitor sequence (AP-1; *lanes 3* and *9*) and 100-fold molar excess of an unrelated unlabeled consensus binding sequence (AP-2; *lanes 4* and *9*). *Lanes 5* and *10* are positive controls for the incubation and gel retardation procedures, and consist of added HeLa cell nuclear extract (Promega, Madison, WI).

matrix and these transcription factor binding sequences may represent tissue-specific complexes of proteins.

DISCUSSION

The nuclear matrix is the framework scaffolding of the nucleus and consists of the peripheral lamins and pore complexes, an internal ribonucleic protein network, and nucleoli. The nuclear matrix has been demonstrated to be an important component of a number of nuclear processes including transcription, replication, and RNA splicing. As described previously, active genes have been found to be associated with the nuclear matrix in cells in which these genes are expressed. In addition, steroid receptors have been shown to bind to the nuclear matrix in a specific fashion. Further investigation into the association of active genes with the nuclear matrix has revealed a DNA loop anchorage site in the enhancer regions or intronic sequences of several genes. These sequences have been termed

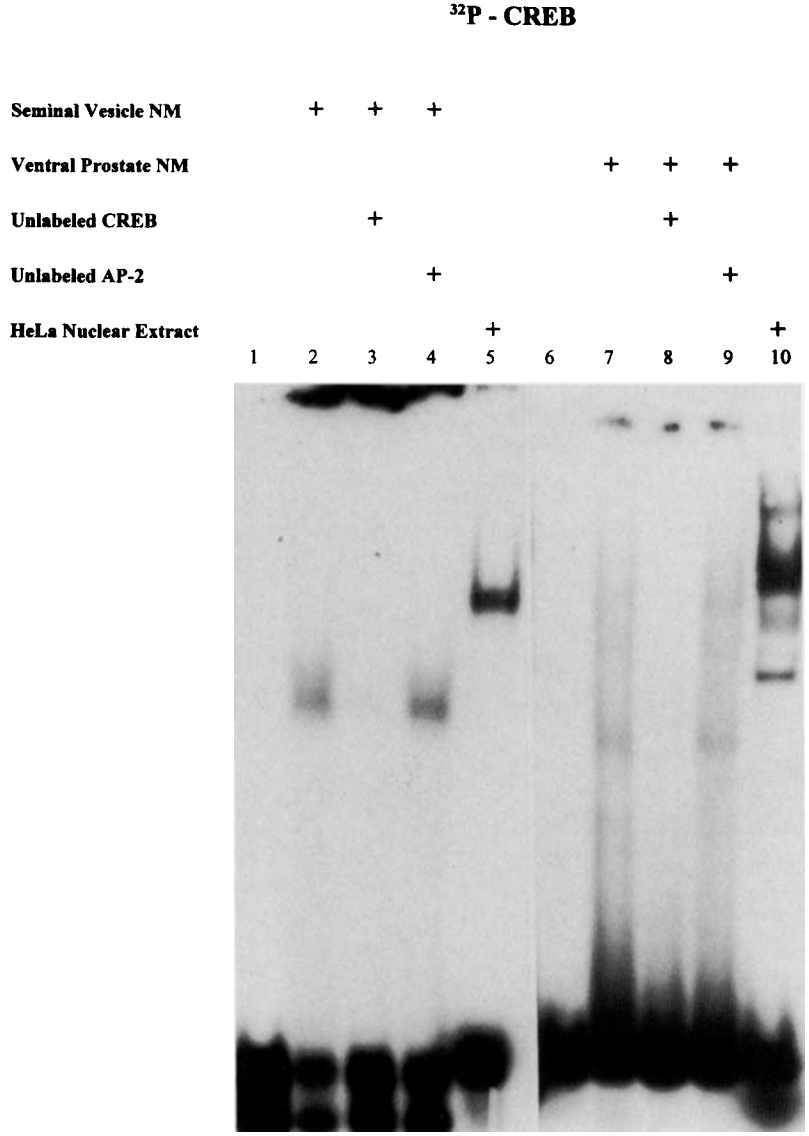

Fig. 6. Presence of CREB binding activity in the nuclear matrix of rat seminal vesicle and ventral prostate. Gel retardation analysis of labeled CREB consensus sequence. *Lanes 1* and *7* are the labeled DNA sequence without the addition of protein (negative control). Ten micrograms of seminal vesicle and ventral prostate nuclear matrix protein (*lanes 2* and *8,* respectively) were incubated with the ^{32}P-labeled cAMP consensus sequence to which CREB binds. Specificity of binding was established by competition of the binding with the addition of 100-fold molar excess of cold competitor sequence (CREB; *lanes 3* and *9*) and 100-fold molar excess of an unrelated unlabeled consensus binding sequence (AP-2; *lanes 4* and *9*). *Lanes 5* and *10* are positive controls for the incubation and gel retardation procedures, and consist of added HeLa cell nuclear extract (Promega, Madison, WI).

matrix-associated regions (MARs) or scaffold-attached regions (SARs), and usually are approximately 200 base pairs in length, are A-T rich, and contain topoisomerase cleavage sequences along with other sequences such as polyadenylation signals [Roberge and Gasser, 1992]. Although these sequences often have a high degree of homology with topoisomerase II cleavage sequences, only one has been found to actually bind topoisomerase II [Sander et al., 1987]. In addition, recent evidence suggests that other sequence types exist for these regions [Roberge and Gasser, 1992]. The MARs have also been shown to functionally confer increased transcriptional activity in transfected genes. In classic experiments, Stief and colleagues inserted the matrix-associated DNA sequences of the chicken lysozyme gene into a transfectable expression vector. When this reporter system was flanked by the 5′ MAR, its expression was markedly

elevated, independent of chromosome position [Stief et al., 1989]. To further examine the role of these MARs in gene expression, experiments were carried out to determine the effects of deletion of MARs on the transcriptional activity of the genes. In the immunoglobulin kappa gene, deletion of the intronic MAR led to a fourfold decrease in expression. When both the intronic MAR and an MAR in the enhancer region were removed from this gene, the expression dropped 11-fold [Blasquez et al., 1989]. These data demonstrate the importance of precise DNA organization for appropriate gene expression.

Recently, nuclear matrix proteins which bind to MAR sequences have been identified by a number of laboratories. These nuclear matrix proteins may serve as the attachment points for these matrix-associated DNA sequences, and it is possible that some of these proteins may be responsible for forming the DNA loop domains. One of these proteins, termed SATB1, is a tissue-specific MAR binding protein, found typically in the thymus. In in vitro cotransfection studies, this protein appeared to act as a transcription suppressor [Dickinson et al., 1992]. Therefore, these proteins, and possibly some of the other MAR binding proteins identified, may be involved in suppressing the expression of specific genes in a tissue-specific manner. Recent investigations have revealed that calmodulin and other nuclear proteins may participate in the association of MARs with the nuclear matrix [Fishel et al., 1993]. Additionally, telomeric DNA sequences have been found to be preferentially associated with the nuclear matrix [de Lange, 1992], indicating that these attachments may be important in DNA organization.

Investigations by a number of laboratories have identified nuclear matrix proteins which are not common to all cell types or conditions. Mitogenic stimulation and the induction of differentiation have been demonstrated to cause alterations in nuclear matrix proteins and structure. Differences in nuclear matrix protein patterns have been noted among various cell lines [Fey and Penman, 1989; Getzenberg and Coffey, 1990], with variations in the nuclear matrix proteins between transformed and nontransformed cell lines of similar origin. The nuclear matrix protein composition is different between normal and transformed cell/tissue types. Our recent evidence indicates that the nuclear matrix of the rat dorsal prostate is different from the nuclear matrix of Dunning prostate tumors, which spontaneously arose from a rat dorsal prostate and between tumors with and without metastatic ability [Getzenberg et al., 1991]. The differences in nuclear matrix protein composition between the normal and tumor cells may play a role in the differences in gene expression found in the transformed state. The nuclear matrix proteins in both human prostate cancer [Partin et al., 1993] and breast cancer [Khanuja et al., 1993] have also been found to differ from normal tissues of the same type.

The presence and absence of nuclear matrix proteins is not the sole mechanism by which nuclear matrix protein composition is regulated. Nuclear matrix proteins may be phosphorylated during growth, at the onset of mitosis, and following androgen treatment [Goueli and Ahmed, 1984]. The regulation of the three-dimensional organization of DNA structure and function appears to be essential to specific gene expression, and the role of tissue-specific nuclear matrix proteins in mediating these changes is important to elucidate.

The data presented in this manuscript provide evidence that the nuclear matrix contains specific components that bind to several transcriptional activator consensus binding sequences. This work supports that of van Wijnen et al. [1993] demonstrating that the nuclear matrix contained sequence-specific DNA binding activities for several transcription factors, although their work examined only one of the activators examined here, AP-1. The associations presented here, between the nuclear matrix and consensus binding sequences for a number of transcriptional activators, suggest the presence of these transcriptional activators on the nuclear matrix.

In addition to the finding that transcriptional activators are associated with the nuclear matrix, we have compared these interactions from various tissue types. Initially, we compared the results found with the rat seminal vesicle nuclear matrix with that of another androgen-responsive tissue, the ventral prostate. In addition to the specific interactions that we identified with the seminal vesicle nuclear matrix, we identified specific interactions with the ventral prostate. These results have demonstrated that the sizes of the retardations of the nuclear matrix with the labeled DNA sequences appear to be different in some instances. This is possibly due to the existence of complexes of nuclear matrix proteins, which include the transcription factor,

that are able to bind to the specific DNA sequences. These complexes may vary from tissue to tissue and possibly may include some tissue-specific components.

HeLa cell nuclear extracts are utilized as positive controls for all studies. The gel shift complexes with nuclear matrix proteins appear, in general, to result in a more diffuse pattern than that observed with HeLa cell nuclear extract complexes. This is possibly due to the fact that the nuclear matrix proteins utilized in these studies are denatured and then renatured as part of the purification procedure. This may affect protein folding for some proteins, possibly resulting in more diffuse patterns. In addition, the patterns representing complexes with nuclear matrix proteins typically have different mobilities than those with HeLa cell nuclear extracts. The HeLa cell nuclear proteins are prepared under different conditions than those utilized here to isolate nuclear matrix proteins. The HeLa cell nuclear extracts are prepared by mild salt extraction of the nuclei whereas the nuclear matrix proteins are prepared by extracting out proteins by relatively stringent salt conditions. Therefore, the complexes of proteins that result from interactions of these HeLa cell proteins may involve protein components that are disassociated by the high salt extraction of the nuclear matrix protein preparation, resulting in some of the smaller complexes identified in these studies.

The evidence provided from these studies furnishes further credence to the possibility that the nuclear matrix serves as a scaffolding structure which brings together specific DNA sequences that are actively expressed in tissues with the components that are necessary to carry out the efficient expression of these genes. Since there are many consensus sequences for transcriptional activators in the genome, few of which are being acted upon by individual transcriptional activators at a single time, the nuclear matrix may provide the mechanism by which these components involved in transcriptional activation are able to associate with the specific DNA sequence and activate gene expression. Therefore, the nuclear matrix as a support structure may provide another level of transcriptional regulation by facilitating the specific interaction of transcription factors with DNA sequences. This level of control may be critical to the cellular regulation of transcription.

ACKNOWLEDGMENTS

This work was supported by financial support from the University of Pittsburgh Cancer Institute, Public Health Service Grant P30 CA47904 from the National Cancer Institute, and Public Health Service Grant DK22000 from the National Institute of Diabetes and Digestive and Kidney Diseases.

We thank Donald Vindivich for assistance with many aspects of this publication.

REFERENCES

Barrack ER (1987): Steroid hormone receptor localization in the nuclear matrix: Interaction with acceptor sites. J Steroid Biochem 27:115–121.

Barrack ER, Coffey DS (1980): The specific binding of estrogens and androgens to the nuclear matrix of sex hormone responsive tissues. J Biol Chem 255:7265–7275.

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

Bidwell JP, van Wijnen AJ, Fey EG, Dworetzky S, Penman S, Stein JL, Lian JB, Stein GS (1993): Osteocalcin gene promoter–binding factors are tissue-specific nuclear matrix components. Proc Natl Acad Sci USA 90:3162–3166.

Blasquez VC, Xu M, Moses SC, Garrard WTJ (1989): Immunoglobulin kappa gene expression after stable integration. I. Role of the intronic MAR and enhancer in plasmacytoma cells. J Biol Chem 264:21183–21189.

Boulikas T, Kong CF (1993): A novel class of matrix attached regions (MARs) identified by random cloning and their implications in differentiation and carcinogenesis. Int J Oncol 2:325–330.

Carter KC, Lawrence JB (1991): DNA and RNA within the nucleus: How much sequence-specific spatial organization? J Cell Biochem 47:124–129.

Carter KC, Bowman D, Carrington W, Fogarty K, McNeill JA, Fay FS, Lawrence JB (1993): A three-dimensional view of precursor messenger RNA metabolism within the mammalian nucleus. Science 259:1330–1335.

Chodosh LA (1988): Mobility shift DNA-binding assay using gel electrophoresis. Curr Protocols Mol Biol 12:2.1–2.10.

Covey L, Choi Y, Prives C (1984): Association of simian virus 40 T antigen with the nuclear matrix of infected and transformed monkey cells. Mol Cell Biol 4:1384–1392.

de Lange T (1992): Human telomeres are attached to the nuclear matrix. EMBO J 11:717–724.

Dickinson LA, Joh T, Kohwi Y, Kohwi-Shigematsu T (1992): A tissue-specific MAR/SAR DNA-binding protein with unusual binding site recognition. Cell 70:631–645.

Eisenman RN, Tachibana CY, Abrams MD, Hann SR (1985): v-myc and c-myc encoded proteins are associated with the nuclear matrix. Mol Cell Biol 5:114–126.

Epperly M, Donofrio J, Barham SS, Veneziale CM (1984): Nuclear protein matrix of seminal vesicle epithelium. J Steroid Biochem 20:691–697.

Fey EG, Penman S (1989): Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc Natl Acad Sci USA 85:121–125.

Fey EG, Bangs P, Sparks C, Odgren P (1991): The nuclear matrix: Defining structural and functional roles. Crit Rev Eukaryotic Gene Expression 1:127–144.

Fishel BR, Sperry AO, Garrard WT (1993): Yeast calmodulin and a conserved nuclear protein participate in the in vivo binding of a matrix association region. Proc Natl Acad Sci USA 90:5623–5627.

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

Getzenberg RH, Coffey DS (1990): Tissue specificity of the hormonal response in sex accessory tissues is associated with nuclear matrix protein patterns. Mol Endocrinol 4:1336–1342.

Getzenberg RH, Coffey DS (1991): Interaction of tissue specific genes with tissue specific nuclear matrix proteins in rat sex accessory tissues. J Cell Biol 115:121A.

Getzenberg RH, Pienta KJ, Huang EYW, Coffey DS (1991): Identification of nuclear proteins in the cancer and normal rat prostate. Cancer Res 51:6514–6520.

Goueli SA, Ahmed K (1984): Phosphorylation of prostatic nuclear matrix proteins is under androgenic control. Arch Biochem Biophys 234:646–650.

Haaf T, Schmid M (1991): Chromosome topology in mammalian interphase nuclei. Exp Cell Res 192:325–332.

Harris SG, Smith MC (1988): SnRNP coreprotein enrichment in the nuclear matrix. Biochem Biophys Res Commun 152:1383–1387.

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

Khanuja PS, Lehr JE, Soule HD, Gehani SK, Noto AC, Choudhury S, Chen R, Pienta KJ (1993): Nuclear matrix proteins in normal and breast cancer cells. Cancer Res 53:3394–3398.

Landers JP, Spelsberg TC (1992): New concepts in steroid hormone action: Transcription factors, proto-oncogenes and the cascade model for steroid regulation of gene expression. CRC Crit Rev Eukaryotic Gene Expression 2:19–63.

Lichter P, Ward DC (1990): Is non-isotopic in situ hybridization finally coming of age? Nature (Lond) 345:93–94.

Lichter P, Cremer T, Tang CJ, Watkins PC, Manuelidis L, Ward DC (1988): Rapid detection of human chromosome 21 aberrations by in situ hybridization. Proc Natl Acad Sci USA 85:9664–9668.

Manuelidis L, Borden J (1988): Reproducible compartmentalization of individual chromosome domains in human CNS cells revealed by in situ hybridization and three dimensional reconstruction. Chromosome 96:397–410.

McNeil JA, Johnson CV, Carter KC, Singer RH, Lawrence JB (1991): Localizing DNA and RNA within nuclei and chromosomes by fluorescence in situ hybridization. Genet Anal Tech Appl 8:41–58.

Nickerson JA, Krochmalnic G, Wan KM, Penman S (1989): Chromatin architecture and nuclear RNA. Proc Natl Acad Sci USA 86:177–181.

Pardoll DM, Vogelstein B, Coffey DS (1980): A fixed site of DNA replication in eukaryotic cells. Cell 19:527–536.

Partin AW, Getzenberg RH, CarMichael MJ, Vindivich D, Yoo J, Epstein J, Coffey DS (1993): Nuclear matrix protein patterns in human benign prostatic hyperplasia and prostate cancer. Cancer Res 53:744–746.

Roberge M, Gasser SM (1992): DNA loops: Structural and functional properties of scaffold-attached regions. Mol Microbiol 6:419–423.

Sander M, Hsieh TS, Udvardy A, Schedl P (1987): Sequence dependence of *Drosophila* topoisomerase II in plasmid relaxation and BND binding. J Mol Biol 194:219–229.

Sarnow P, Mearning P, Anderson CW, Reich N, Levine AJ (1982): Identification and characterization of an immunologically conserved adenovirus early region 11,000 M_r protein and its association with the nuclear matrix. J Mol Biol 162:565–583.

Schuchard M, Subramaniam M, Ruesnick T, Spelsberg TC (1991a): Nuclear matrix localization and specific matrix DNA binding by receptor binding factor-1 of the avian oviduct progesterone receptor. Biochemistry 30:9516–9522.

Schuchard M, Rejman JJ, McCormick DJ, Gosse B, Rusenick T, Spelsberg TT (1991b): Characterization of a purified chromatin acceptor protein (receptor binding factor-1) for the avian oviduct progesterone receptor. Biochemistry 30:4535–4542.

Spector DL (1990): Higher order nuclear organization: Three-dimensional distribution of small nuclear ribonucleoprotein particles. Proc Natl Acad Sci USA 87:147–151.

Staufenbiel M, Deppert W (1983): Different structural systems of the nucleus are targets for SV40 large T antigen. Cell 33:173–181.

Stief A, Winter DM, Stratling WH, Sippel AE (1989): A nuclear DNA attachment element mediates elevated and position-independent gene activity. Nature (Lond) 341: 343–345.

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

van Wijnen AJ, Bidwell JP, Fey EG, Penman S, Lian JB, Stein JL, Stein GS (1993): Nuclear matrix association of multiple sequence-specific DNA binding activities related to SP-1, ATF, CCAAT, E/EBP, OCT-1 and AP-1. Biochemistry 32:8397–8402.

Xing Y, Johnson CV, Dobner PR, Lawrence JB (1993): Higher level organization of individual gene transcription and RNA splicing. Science 259:1326–1330.