DNA-BINDING PROPERTIES OF NUCLEAR MATRIX PROTEINS

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SUMMARY

Mouse nuclear matrix proteins, examined by a filter assay, were found to bind to DNA. There was no preference for homologous mouse compared to heterologous E. coli DNA. Competition assays showed a preference for AT-rich DNA and of the 4 single-stranded homopolymers there was a preference for poly(dT). These observations are consistent with the possibility that the matrix may play a role in the formation of AT-rich chromomeres (G-bands).

INTRODUCTION

The nuclear matrix provides the architectural framework of the nucleus and is composed of a nuclear pore-lamina complex, intranuclear matrix, and nucleolar matrix (Aaronson & Blobel, 1975; Berezney & Coffey, 1974, 1976; Comings & Okada, 1976; Riley, Keller & Byers, 1975). It is composed primarily of 3 major polypeptides of 65,000, 67,000 and 68,000 Daltons molecular weight, as determined by SDS acrylamide gel electrophoresis. Included in the matrix are 2-nm fibrils, previously termed matrixin (Comings & Okada, 1976), which may associate to form larger fibres. Many studies have shown that chromatin is not free in the nucleus but is attached at multiple sites to the inner nuclear membrane (Brasch, Seligy & Setterfield, 1971; Comings, 1968, 1972; Comings & Okada, 1970a, b, 1971; DuPraw, 1965; Franke & Scheer, 1971). Because of this association we have examined the DNA-binding properties of nuclear matrix proteins. We find they bind to both homologous and heterologous DNA and show preferential binding to AT-rich DNA.

MATERIALS AND METHODS

Preparation of 14C- and 3H-labelled DNA

For labelled mouse DNA, Krebs ascites cells were incubated with gentle rocking in McCoy’s media with 10% foetal calf serum, 1% non-essential amino acids, penicillin and streptomycin. The cells were incubated with 5 μCi/ml of [3H]thymidine (40–60 Ci/mm, New England Nuclear) or 0.1 μCi/ml [14C]thymidine (40–60 mCi/mm) for 12–14 h.

For labelling E. coli DNA, thymidine-1G 108 E. coli were grown with gentle rocking in Media 9 (Miller, 1972) plus 0.4% glucose and 0.5 μg/ml cold thymidine for 20–24 h at 37 °C. This was then diluted with the same media to an O.D. 600 nm of 0.4–0.6, either 0.5 μCi/ml of [14C]thymidine or 5 μCi/ml of [3H]thymidine added, and incubation continued for an additional 12–14 h.
DNA isolation

The DNA was isolated by a modification of the Marmur (1961) procedure. Aliquots of 500 µg of DNA were centrifuged to equilibrium in cesium chloride, the tubes fractionated and the DNA peak dialysed against multiple changes of TN buffer (0.1 M NaCl, 0.1 mM EDTA, 10 mM Tris, pH 7.6) for 48 h.

After dialysis the DNAs were sheared by passing through a no. 25 needle 3 times. The molecular weight of the sheared DNA was determined by sedimentation velocity using adsorption optics of the Model E analytical ultracentrifuge by the technique of Doty, McGill & Rich (1958). DNAs sheared to a molecular weight of 4–5 × 10^6 Daltons were used.

DNA-binding assays

To assay protein-DNA binding the nitrocellulose filter assay of Riggs, Bourgeois, Newby & Cohn (1968) and Riggs, Suzuki & Bourgeois (1970) was used. Stock solutions of DNA and protein were kept in TN buffer (0.1 M NaCl, 0.1 mM EDTA, 0.01 M Tris-HCl, pH 7.5). The protein concentration was determined by TCA assay (Comings & Tack, 1972). The final binding assay consisted of 0.5 µg of 1^4C-DNA with a specific activity of 1500 to 2000 cpm, 0.5 µg 3H-DNA with a specific activity of 5000 to 6000 cpm, and 0 to 30 µg of nuclear matrix protein, in 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.5, 0.1 mM EDTA, 3 mM MgCl₂, 0.1 mM DTT in a total volume of 0.7 ml. The 2 DNAs were first added to small test tubes and brought to 3 mM with MgCl₂ and 0.1 mM with DTT. Then variable amounts of buffer A (0.1 M NaCl, 0.01 M Tris-HCl, pH 7.5, 0.1 mM EDTA, 3 mM MgCl₂, 0.1 mM DTT) were added so that the final volume was 0.7 ml. Appropriate amounts of protein solution, which had been brought to 3 mM MgCl₂ and 0.1 mM DTT were then added, gently vortexed and the mixture allowed to sit at room temperature for 10 min. The sample was then layered onto 25-mm HAWP o.45-µm Millipore filters presoaked in buffer A. The solution was exposed to gentle suction requiring 5–10 s to filter. The filter was then washed 3 times with 0.5 ml of buffer A. The filters were placed in scintillation vials, allowed to dry until just moist and 10 ml of scintillation mixture added (5 g PPO, 100 g naphthalene/1, dioxane). The vials were counted in a Beckman Model 150 scintillation counter and counts corrected for 3H and 14C spillover with a computer programme. Each point was done in duplicate. To determine the amount of radioactivity added to each vial a solution containing 0.5 µg of each DNA was added to a scintillation vial with a moist Millipore filter and counted. This was done in duplicate. The background binding was determined from a sample to which no protein had been added. This usually ranged from 1 to 8% DNA retained. This background was subtracted from each sample containing protein.

For competition assays, a constant amount of protein and 1 µg of 3H-labelled mouse DNA were mixed with 0 to 40 µg of competing cold DNA.

Isolation of nuclear matrix

Mice were killed by cervical dislocation and the livers cut into pieces in TCMB (0.01 M Tris-HCl, 0.1 mM cadmium sulphate, 3 mM magnesium chloride, 1 mM sodium bisulphite, 1 µg/ml soybean trypsin inhibitor, pH 7.0). Nuclei were isolated from the livers of 40 mice as described previously (Comings & Harris, 1975). The nuclear matrix was isolated by a modification of the technique of Berezney & Coffey (1974). The nuclei were washed once in 40 ml of 0.05 M Tris, 0.2 mM MgCl₂, 1 µg/ml soybean trypsin inhibitor with a 500-g, 5-min centrifugation. The pellet was then washed twice in 0.15 M NaCl, 0.2 mM MgCl₂, 0.01 M Tris, pH 7.5 again with 500-g, 5-min centrifugations. The pellet was resuspended in 10 ml of the same buffer and placed in a 300-ml beaker with a stirring bar in the cold room. Two hundred millilitres of 2 M NaCl, 0.01 M Tris, 0.2 mM MgCl₂, pH 7.5, were added slowly with stirring and the mixture stirred for an additional 5 min. This was then distributed into four 50-ml plastic Sorvall tubes and centrifuged at 15 000 g for 10 min. The pellet was resuspended in 40 ml of the same buffer and centrifuged at 1000 g for 10 min. The pellet was then washed once in 0.01 M Tris, 5 mM MgCl₂, 1% Triton X-100, pH 7.5, with centrifugation at 1000 g for 10 min. The pellet was resuspended in 5 ml of 0.01 M Tris, 5 mM MgCl₂, pH 7.5 + 200 µg/ml of DNase I (Sigma) and 200 µg/ml of RNase A (Sigma). This was incubated at room temperature for 30 min and then centrifuged at
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1000 g for 10 min. The pellet was washed twice in 2 M NaCl buffer (above) and twice in distilled water. This pellet was resuspended in 5 ml of 2 M NaCl, 5 M urea, 0-01 M Tris, pH 7.5, and passed through 3 freeze-thaw cycles. This freeze-thawing breaks hydrophobic bonds and breaks up the nuclear matrix. This suspension was centrifuged at 10000 g for 10 min and the supernatant dialysed against 2 l. of TN buffer overnight. This was then centrifuged at 5000 g for 10 min. The 10–20% of the protein remaining in suspension was considerably enriched in bands C10, D1 and D4 (Fig. 2, p. 237) (Comings & Harris, 1975; Comings & Okada, 1976). Freeze-thawing in 1 M guanidine thiocyanate and dialysis against TN buffer gave the same results.

The cytoplasmic saline-EDTA, Tris and 0.35 M NaCl nuclear wash proteins were isolated as described previously (Comings & Harris, 1975).

All synthetic polynucleotides were obtained from Miles Laboratories, Elkhart, Ind. The bacterial DNAs were obtained from Sigma, St Louis.

**RESULTS**

**DNA binding of nuclear wash proteins**

To obtain a frame of reference for evaluating the DNA-binding capabilities of nuclear matrix proteins, DNA-binding curves for other non-histone proteins were examined. These proteins were obtained by successively washing mouse liver nuclei with saline-EDTA, dilute Tris, and 0.35 M NaCl (Comings & Harris, 1976). When these washes were centrifuged at 100000 g for 1 h, the supernatants were devoid of histones. Cytoplasmic proteins were also examined. The cytoplasmic proteins showed the least binding (30%), and saline-EDTA was next (42%), then the Tris-wash proteins (50%), and finally the 0.35 M NaCl wash proteins showed the greatest binding (65%) (Fig. 1). To examine the DNA-binding capacity of high-molecular-weight fibrillar proteins, mouse F-actin was tested. At 10 µg protein it showed less than 5% DNA retained. For comparison with histones, whole calf thymus histones were used in filter-binding assays. These resulted in 100% retention of DNA at 0.5 µg of protein.

**DNA binding of nuclear matrix proteins**

The nuclear matrix proteins used in the binding assays are shown in Fig. 2. By SDS gel electrophoresis they consist of 3 bands, C10, D1 and D4 (Comings & Harris, 1975, 1976; Comings & Okada, 1976) at molecular weights of 68000, 67000 and 65000 Daltons. They were completely free of histones. In all assays the proteins were prepared on day 1, and after dialysing overnight against TN buffer they were used in binding assays on day 2 and sometimes on day 3. Older preparations showed decreased DNA binding and were not used. Fig. 3 illustrates an example of the optimal results.

The matrix showed a high affinity for DNA with up to 100% of the DNA bound at 5 to 10 µg of protein added. Similar results were obtained when the nuclear matrix was disrupted by freeze-thawing in 1 M guanidine thiocyanate and dialysed against TN buffer. Guanidine thiocyanate is a strongly chaotropic buffer (Hatefri & Hanstein, 1974). The affinity of nuclear matrix proteins for DNA is high but not as great as that of histones (100% retention of 0.5 µg protein). The nuclear matrix proteins, however, are acidic proteins with a ratio of acidic to basic amino acids of 1.4 and an isoelectric point of the different components ranging from 4.7 to 6.5 (Comings, unpublished data).
Fig. 1. DNA binding by cytoplasmic proteins (d) and proteins in the saline-EDTA (c), Tris (b) and 0.15 M NaCl (a) washes of isolated nuclei.

Not all preparations showed this degree of DNA retention. The usual range was 50 to 100% DNA retained at 10 μg protein added.

The stoichiometry of the matrix binding is difficult to determine because of the aggregative nature of these hydrophobic proteins. The freeze-thaw treatment in 2 M NaCl, 5 M urea followed by dialysis to 0.1 N NaCl and centrifugations (see Methods) purifies and disperses the 65,000, 67,000 and 68,000 Dalton polypeptides but does not solubilize them. Under phase microscopy, macromolecular protein complexes are visible. Under whole-mount electron microscopy fibrillar proteins like those described previously (Comings & Okada, 1976) are visible. At an absolute minimum of one each
of the polypeptides in a complex, the molecular weight would be \(2 \times 10^6\) Daltons. Presumably it is much higher. The molecular weight of the DNA was \(4 \times 10^6\) Daltons and in optimal preparations \(0.5 \mu g\) of DNA or \(7.5 \times 10^6\) molecules were retained by \(2 \mu g\) of matrix protein. With a minimum weight of the complex of \(2 \times 10^6\) Daltons, \(2 \mu g\) represent \(6.0 \times 10^{12}\) molecules or 80 molecules of matrix per molecule of DNA bound. With higher, more reasonable estimates of the molecular weight of the complex, this could range down as low as one matrix complex per DNA molecule retained. It is presumably a variation in the degree of aggregation of the hydrophobic matrix proteins, giving a variation in the effective protein concentration, which accounts for the variability in DNA retained/\(\mu g\) of matrix protein added.

In these experiments in which both \(^{14}C\) mouse and \(^3H\) \(E. coli\) DNA were included in the same binding assay, there was no significant preference for homologous DNA. This was confirmed by competition experiments using unlabelled DNA (Fig. 4). Here, \(2 \mu g\) of matrix protein were used in each assay and \(1 \mu g\) of \(^3H\) mouse DNA was competed out with up to \(40 \mu g\) of cold mouse or \(E. coli\) DNA. At \(5 \mu g\) of cold mouse DNA the DNA retained had dropped to \(0.57\) of control levels, at \(5 \mu g\) of cold \(E. coli\) DNA

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**Fig. 2.** SDS gel electrophoresis of the nuclear matrix proteins used in the binding assay. C10, D1, and D4, 68,000, 67,000 and 65,000 Daltons, respectively.
the % DNA retained had dropped to 0.61 of control levels, indicating little preferential binding to homologous DNA.

**Matrix proteins preferentially bind to dT-rich single-stranded DNA**

Since mouse and *E. coli* DNA have a similar mean base composition, some additional DNAs were used in competition experiments to determine if nuclear matrix protein showed a preference to AT or GC-rich DNA. As shown in Fig. 5, there was somewhat
improved competition with *Cl. perfringens* DNA of 70% AT content. Here the DNA binding in the presence of 5 μg of competitor DNA was 0.48 of the control. By contrast, competition with 5 μg of *M. lysodeikticus* DNA, of only 29% AT content, decreased binding to only 0.7 of the control.

To examine further the role of base composition poly(dA-dT) (Fig. 6) and poly(dG)·poly(dC) (not shown) were used in competition experiments. With 5 μg of poly(dA-dT) the % DNA retained was 0.4 of the control, with poly(dG)·poly(dC) the comparable
Fig. 6. Competition of cold poly(dA-dT), poly(dG), poly(dA) and poly(dT) with 1 µg of ³H mouse DNA for 10 µg of mouse nuclear matrix protein. Arrows = 5/o value.

Fig. 7. Plot of 5/o values (see text) against % AT content of competing cold DNA.

value was 0.85. These values of % DNA retained with 5 µg of competitor DNA/% retained without competitor DNA (5/o value) are plotted against base composition of competitor DNA in Fig. 7. This indicated that AT-rich double-stranded DNA binds to nuclear matrix DNA more effectively than GC-rich DNA.

Comparison of double-stranded to single-stranded mouse DNA indicated single-stranded DNA is by far the more efficient competitor (Fig. 5). A control experiment using filters preincubated with 10 µg of single-stranded DNA indicated the decreased binding was not simply due to clogging up the filters with single-stranded DNA.

On the basis of the above results one would anticipate that single-stranded poly(dA)
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or poly(dT) DNA would bind nuclear matrix very effectively. To examine this the
4 polydeoxynucleotides were compared as competitors for double-stranded \(^3\)H mouse
DNA. As shown in Fig. 6, poly(dA) was the least effective competitor while poly(dT)
was the most effective. At 1 \(\mu g\) of poly(dT) the % DNA bound had dropped to 16%
of the control value, and with 5 \(\mu g\) it was 10% of the control value. The 5/0 value for
poly(dA) was 0.6. Poly(dG) (0.25) was the same as poly(dC) (not shown). In additional
experiments the 5/0 value for poly(dT) was 0.10 and the values for poly(dA) were
0.65 and 0.85. Because of its preference for single-strand DNA, it was of interest to
determine whether the nuclear matrix proteins are capable of denaturing DNA in the
absence of cation as protein 32 does (Alberts & Frey, 1970). Ten microgrammes of
poly(dA-dT) were mixed with 75 \(\mu g\) of nuclear matrix protein, for a protein/DNA ratio
of 7.5, and the O.D. 260 nm followed with time. As shown in Fig. 8, there was a significant
increase in O.D. 260 nm following a marked instantaneous rise. However, since
this was producing an increase in O.D. 260 nm far in excess of that produced with heat
or alkali, it was unlikely that the increase was due to denaturation. The solution showed
slight turbidity and a spectral curve from 325 to 220 nm confirmed that the increased
O.D. 260 nm was due to light scattering from aggregation of the DNA by nuclear
matrix. These results also differed from those with protein 32 in that addition of Mg\(^{2+}\)
exacerbated rather than reversed the increase in O.D. 260 nm. This marked aggrega-
tion of DNA is consistent with the DNA-binding properties of the nuclear matrix
proteins.

In view of the preferential binding of matrix proteins to poly(dT) over poly(dA), one
would anticipate that the binding to poly(dA)-poly(dT) would be greater than the

Fig. 8. Three cuvettes were loaded with 10 \(\mu g\) of poly(dA-dT) at 37 °C in TN buffer
and at time 0, 75 \(\mu g\) of nuclear matrix protein were added to 2 cuvettes, one of which
also contained 0.01 M MgCl\(_2\). The addition of nuclear matrix resulted in immediate
aggregation of the poly(dA-dT) with increase of the O.D. 260 nm. This aggregation
slowly increased over the subsequent 30 min. At 25 min the cuvette without MgCl\(_2\)
was brought to 0.05 M MgCl\(_2\). This resulted in accelerated aggregation of DNA.
binding to poly d(A-T) since the former would have longer stretches of poly(dT). As shown in Fig. 9, this was the case, poly(dA)·poly(dT) competed with \(^3\)H mouse DNA significantly better than poly d(A-T). When 5 \(\mu\)g of nuclear matrix protein were added to 20 \(\mu\)g of poly(dA)·poly(dT) in TN buffer, for a protein/DNA ratio of 0.25, and incubated at 37 °C it caused no increase in O.D. 260 nm over a 2-h time span. Thus, under these conditions there was no denaturation of poly(dA)·poly(dT).

![Graph](image)

Fig. 9. Competition of cold poly(dA-dT) and poly(dA)·poly(dT) with 1 \(\mu\)g of \(^3\)H mouse DNA for nuclear matrix protein. The poly(dA)·poly(dT) is a more efficient competitor than poly(dA-dT).

To examine the ionic sensitivity of the matrix-DNA complex, the DNA binding of matrix at various NaCl concentrations was examined. This was done in 2 ways. In one experiment the matrix and DNA were mixed in TN buffer of varying salt concentrations, then after 10 min placed on filters. In the second experiment they were mixed together in the usual 0.1 M NaCl TN buffer, then after 10 min the NaCl concentration was increased to the levels indicated and incubated for an additional 10 min. Both experiments gave the same results. Binding was maximum at 0.1 to 0.2 M NaCl then decreased rapidly by 0.5 and 1.0 M NaCl.

**DISCUSSION**

The nuclear matrix is prepared from isolated nuclei by gentle extraction of all the chromatin with high salt and nucleases, and removal of the outer membrane with Triton X-100 (Berezney & Coffey, 1974). This results in a structure which, by phase
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microscopy, is very similar to the original nucleus. It is composed of a nuclear pore-lamina complex (Aaronson & Blobel, 1975), internal nuclear matrix and nucleolus matrix (Berezney & Coffey, 1974; Comings & Okada, 1976). Although it has been suggested that the lamina layer lies beneath the inner nuclear membrane (Aaronson & Blobel, 1975; Dwyer & Blobel, 1976) it may also be a remnant of the inner nuclear membrane altered by the salt and Triton X-100 treatment (Scherrer et al., 1976). Amino acid analysis of the matrix proteins (Comings, 1978) show they are very similar to red blood cell membrane proteins by the SAD test (Weltman & Dowben, 1973). SDS gel electrophoresis shows predominantly 3 polypeptides of 68,000, 67,000 and 65,000 Daltons (Aaronson & Blobel, 1975; Berezney & Coffey, 1974; Comings & Okada, 1976). At least a portion of the matrix is composed of 2-nm protein fibres which may associate to form larger fibres (Comings & Okada, 1976; Scherrer et al., 1976).

Examination of the matrix proteins by filter-binding assay shows they have a high affinity for DNA. They show no significant preference for homologous mouse DNA compared to heterologous E. coli DNA. This concurs with studies of Rowekamp & Sekeri (1974) who found that rat nuclear membrane proteins isolated by a phenol procedure had a high affinity for both homologous and heterologous DNA, based on a sucrose gradient analysis. Competition experiments with DNAs of varying base composition indicates the matrix proteins have a preference for single-stranded DNA. Use of single-stranded homopolymers in the competition assays indicated a much greater affinity for poly(dT) compared to poly(dA). Binding to poly(dG) and poly(dC) was intermediate between poly(dT) and poly(dA). Despite a preference for single-stranded DNA, under the conditions used, nuclear matrix protein does not cause denaturation of poly(dA) • poly(dT) as does gene 32 protein (Alberts & Frey, 1970).

Some potential problems with these studies should be emphasized. (1) Since some DNA remains associated with the nuclear matrix even after several extractions with 2 M NaCl, the matrix-DNA bonds in vivo appear to be very tight. By contrast the isolated nuclear matrix that was rebound to the DNA was quite sensitive to salt concentrations greater than 0.5 M. This suggests that the filter-binding assay does not completely reproduce all the in vitro factors involved in the binding of matrix to DNA. (2) The freeze-thawing in high salt releases only a portion of the nuclear matrix proteins. Although these were the major matrix polypeptides (Berezney & Coffey, 1976; Aaronson & Blobel, 1975; Comings & Okada, 1976), some protein participating in the DNA binding may have been excluded. (3) Binding to AT-rich DNA may be a non-specific property of many proteins and not be physiologically significant. For example H1 histone shows a preference for AT-rich DNA (Sponar & Sormova, 1972) but this is probably not of physiological importance. Catino, Yeoman & Busch (1977) have described a rat liver non-histone protein that also shows preference for AT-rich DNA.

Matrix, membranes and DNA replication

In the studies by Aaronson & Blobel (1975) the 65,000, 67,000 and 68,000 Dalton peptides were present in a lamina-pore complex isolated from the nuclear membrane. In the isolation procedure used in our studies these proteins were found to have a
wider distribution, being present in a nuclear matrix distributed throughout the nucleus as well as at the nuclear membrane (Berezney & Coffey, 1974, 1975; Comings & Okada, 1976; Comings, 1978).

The amino acid composition of whole nuclear matrix (Comings, 1978) was compared to many proteins by the SÄQ test (Weltmann & Dowban, 1973). It was more closely related to red cell membrane proteins than to some 25 other proteins examined.

Berezney & Coffey (1975) have recently shown that nascent DNA is preferentially associated with the nuclear matrix protein. Although autoradiography studies indicate that the replication fork is not associated with the nuclear membrane associated matrix (Comings & Okada, 1973; Huberman, Tasi & Deich, 1972; Williams & Ockey, 1970; Wise & Prescott, 1973) the replication fork may be associated with the intranuclear matrix.

Relationship to chromomere structure

Chromatin has many levels of order including the nucleosome, the chromomeres of dipteran polytene chromosomes, the chromomeres of pachytene chromosomes, chromosome coiling and the chromosome itself. The fundamental nature of the pachytene chromomeres is indicated by the fact they correlate precisely with the G-bands of mitotic chromosomes (Ferguson-Smith & Page, 1973; Okada & Comings, 1974; Luciana, Devictor, Morazzani & Stahl, 1976). The fact that G-banding can be produced even after all the histones have been removed by HCl (Comings & Avelino, 1975) suggests the chromomere arrangement may be secondary to an interaction between DNA and a non-histone protein. Since the G-bands were relatively AT-rich (Comings, 1973; Comings & Drets, 1976; Schreck et al., 1973; Weisblum & de Haseth, 1972) we were especially interested in whether nuclear matrix protein might show a specific affinity to certain DNA sequences. The lack of preference for homologous over heterologous DNA made this unlikely. There was, however, a significant preference for AT-rich DNA. Studies of daunomycin-DNA fluorescence suggest the G-bands may contain regions of very AT-rich DNA (Comings & Drets, 1976). The binding of such regions to nuclear matrix protein could be responsible for production of chromomeres.

The marked preference of nuclear matrix for single-stranded poly(dT) strands was of interest. This preference does not prove that matrix only binds to single-stranded DNA in vivo. During some stages of the cell cycle there appears to be no single-stranded DNA (Collins, Berry & Coffs, 1977).

In conclusion, the remarkable association between DNA and the nuclear matrix as shown by thin section and whole mount electron microscopy (Berezney & Coffey, 1976; Comings & Okada, 1976) raised a number of questions about the nature of this interaction. Are specific DNA sequences involved or is the interaction totally non-specific, similar to that of histones and DNA? The insoluble, hydrophobic, aggregating nature of the matrix proteins made investigation of such questions difficult. Freeze-thawing in 2 M NaCl, 5 M urea followed by dialysis to lower the salt content and remove the urea, allowed dispersion of the matrix. Since this did not solubilize the matrix, standard DNA affinity columns could not be used. Filter-bindings assays,
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however, were found to be suitable and provided evidence for the high capacity for matrix proteins to bind DNA. This was verified by the strong tendency for matrix proteins to aggregate DNA as followed by spectrophotometry. Competition assays indicate the matrix proteins preferentially bind to the poly dT-rich strand of AT-rich DNA.

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REFERENCES


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