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DNA–Protein Interactions of the Rat Liver Non-Histone Chromosomal Protein†

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ABSTRACT: Native rat liver NHC protein–DNA interactions have been investigated by use of a nitrocellulose filter assay sensitive in detection of protein–DNA complexes. Optimal conditions for DNA–protein interactions occurs at low ionic strength conditions (110 mM–0.04 M phosphate buffer). A fraction of NHC proteins was enriched 25-fold by their affinity for rat DNA immobilized on cellulose columns under these conditions. At higher ionic strength (260 mM–0.04 M phosphate buffer and 0.15 M sodium chloride), this fraction binds approximately sevenfold less to rat DNA but with a substantial increase in stability of the complexes. Equilibrium competition experiments indicate that at the higher ionic strength there is a considerable DNA sequence specificity of the rat DNA binding NHC protein. Since rat DNA contains three components as defined by their reassociation kinetics: single copy DNA ($C_0t_{1/2}^{pure} = 1.6 \times 10^3$); middle repetitive DNA ($C_0t_{1/2}^{pure} = 1.1$); and highly repetitive ($C_0t_{1/2}^{pure} < 0.02$). The two former were isolated and employed in the DNA binding assays. At the high ionic strength criterion, the rat DNA binding NHC proteins showed a substantial preference for a subset of middle repetitive DNA sequences. This suggests a preferential interaction between a class of NHC proteins and a class of middle repetitive DNA sequences.

Rat liver non-histone chromosomal proteins (NHC proteins) can be fractionated into three groups by tandem DNA-cellulose column chromatography (van den Brock *et al.,* 1973). The column series consisted of an *Escherichia coli* DNA-cellulose column connected to a rat DNA-cellulose column. The groups consist of NHC proteins which do not bind to either DNA-cellulose column, proteins which bind to the *E. coli* DNA column, and a third group which bind to the rat DNA-cellulose column. The present paper analyzes in more detail the specificity of the rat DNA binding NHC proteins by use of the nitrocellulose filter assay

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system for the detection of protein-DNA complexes (Riggs *et al.*, 1968; Riggs and Bourgeois, 1968). We show below that the rat DNA-binding proteins of rat liver chromatin bind preferentially to rat DNA with a preference for the middle repetitive segments of the rat genome.

Methods

Preparation and Fractionation of the Non-Histone Chromosomal Proteins. NHC proteins were prepared from purified chromatin (Bonner *et al.*, 1968) of male rat livers (Pel Freeze Biologicals, Rogers, Ark.) as described previously (van den Broek *et al.*, 1973) with one major modification. After the chromosomal proteins were dissociated from DNA in 4 M sodium chloride–10 mM Tris (pH 8.0), DNA was removed by centrifugation for 20 hr at 215,000*g* in a Beckman Spinco Ti-60 rotor. A yield of 83.3% ± 11.1% of the original chromosomal proteins was obtained (eight preparations). Bio-Rex 70 ion exchange chromatography was used to remove the histones from the NHC proteins (Levy *et al.*, 1972). From 3000 OD_{260} (144 mg of DNA) units of dissociated chromatin a yield of 45–50 mg of NHC proteins was obtained. The NHC proteins were fractionated by their DNA binding characteristics over a tandem series of DNA-cellulose columns (Alberts and Herrick, 1971). By this procedure, we can enrich by 25-fold a fraction of NHC proteins which bind preferentially to the second DNA-cellulose column (rat DNA).

Isolation of DNA and Fractionation of DNA Components. DNA was prepared from *Micrococcus lysodeikticus*, calf thymus (Sigma Chemical Company, St. Louis, Missouri), *Drosophila melanogaster* embryos, *Dictysotelium discoideum*, *E. coli B* cells, and frozen rat livers (General Biochemicals) by the procedure of Marmur (1961), and of Wu *et al.* (1972).

Rat ascites [^{3}H]DNA (~10,000 cpm/μg of DNA) were prepared from rat ascites cells grown *in vivo* injected with 1 mCi per rat of [^{3}H]thymidine (Schwarz/Mann, Orangeburg, N.Y.) (specific activity 17 Ci/mmol) 48 hr before harvesting (Dahmus and McConnell, 1969). All native DNAs employed in the protein binding and competition assays were sheared to a number average 650 ± 100 base pairs (as judged by electron microscopy) by passage through a Ribi-Sorvall cell fractionator three times at 30,000 psi. The sheared DNA was phenol-extracted and dialyzed against 0.12 M phosphate buffer (pH 6.8), and then passed over hydroxylapatite at 62° (Britten and Kohne, 1966). Double-stranded DNA was eluted with 0.48 M phosphate buffer and was >95% of the input DNA. The thermal denaturation profile in 0.12 M phosphate buffer of this DNA is characterized by a hyperchromicity of 33.7%. Such DNAs melt with a T_m of 80–85° (depending on DNA) over a range of 10°.

Rat DNA (labeled or not labeled) was fractionated into its middle repetitive and single copy components essentially by the methods outlined in Holmes and Bonner (1974). DNA (1 mg/ml) was dissolved in 0.12 M phosphate buffer and sheared to 350 nucleotides (as judged by electron microscopy) by three passages through a Ribi-Sorvall cell fractionator at 50,000 psi. The components were prepared by incubation of denatured DNA in phosphate buffer to an appropriate equivalent C_0t followed by separation of double- from single-stranded DNA by hydroxylapatite chromatography (Britten and Kohne, 1967). Single copy DNA was isolated by incubating denatured DNA first to a C_0t of 9×10^2 during a first cycle of reassociation where 60% of the DNA was single stranded and then to a C_0t of 10^3 on a second cycle of reassociation. The single-stranded DNA isolated by these two fractionations were reassociated by incubation in 0.48 M phosphate buffer (pH 6.8) until more than 90% was double stranded. Middle repetitive DNA was isolated by separation of DNA that reassociated between C_0t 2×10^{-2} and C_0t 2×10^2. High repetitive DNA, which reassociated before C_0t 10^{-2} (10% of the total), was not employed in these studies.

Since separation of the middle repetitive and single copy sequences involves denaturation of the DNA and subsequent renaturation to selected C_0t's, the reannealed fragments showed a hyperchromicity of 25.6 and 26.5%, respectively. This decrease in hyperchromicity is related to single-stranded ends and mismatched base pairs in the reassociated fragments (Britten *et al.*, 1974). The use of the nitrocellulose filter assay requires DNA with little or no single-stranded ends to keep the nonspecific retention of DNA at a low level. Digestion of the reassociated fragments by purified S1 nuclease of *Aspergillus orzyae* (St. John *et al.*, 1974) under conditions where single-stranded ends were selectively digested resulted in DNA fragments with a double strandedness of 95% as measured by their hyperchromicity. Measurement of the size of the single-stranded pieces of DNA by sedimentation in alkaline solution revealed a preponderance of a single sized species of around 200 bases (Studier, 1965). The number average base pair length was determined by electron microscopy to be 250–260 bp.

Membrane Filter Assay for DNA Binding. Protein–DNA complexes are bound to nitrocellulose filters (Jones and Berg, 1966; Freeman and Jones, 1967; Riggs *et al.*, 1968; Riggs and Bourgeois, 1968). B6 membrane filters (Schleichter and Schuell, Inc.) were soaked in the assay buffer (0.04 M phosphate buffer (pH 6.8)–0.1 mM dithiothreitol with or without NaCl added). The DNA-binding proteins and DNA (1.0 μg/ml) were mixed in a volume of 1.5 ml of assay buffer and allowed to equilibrate at 0° for 15 min. Triplicate, 0.5-ml aliquots were filtered at a rate of 20 sec/sample over individual membrane filters, washed with 1 ml of assay buffer, and counted in a Beckman scintillation spectrometer in aquasol.

Competition Experiments. The preference of proteins for a particular species of DNA may be determined by the equilibrium competition technique (Riggs *et al.*, 1970; Lin and Riggs, 1972). Unlabeled competitor DNA was mixed in a total volume of 1.0 ml with 1.0 μg of ^{3}H-labeled rat DNA and enough NHC protein was added to bind 50% of the labeled DNA (in the absence of competitor). After a 15-min incubation period triplicate samples were filtered over individual membrane filters and washed as above. The results were expressed as the ratio of the amount of labeled DNA retained on the filter in the presence of unlabeled competitor DNA to the amount of labeled DNA retained on the filter in the absence of any competitor. Triplicate determinations were in all cases within 5% of one another. Competition studies with several preparations of DNA-binding NHC proteins yielded competition curves similar to one another within 10%.

General Methods. Protein concentrations were determined by the method of Lowry *et al.* (1951) or by the filter method of Kuno and Kihara (1967) with bovine serum albumin as standard. DNA concentrations were determined spectrophotometrically using an extinction coefficient of 6600 (liters per gram centimeter).

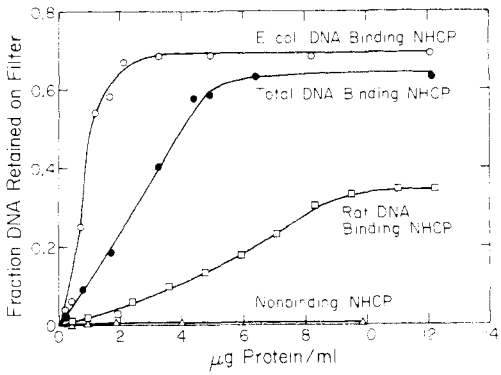

FIGURE 1: DNA saturation with increasing NHC protein. Increasing amounts of total NHC proteins (●), *E. coli* DNA binding NHC proteins (○), rat DNA binding NHC proteins (□), and nonbinding NHC proteins (△) were mixed with 1.0 μg/ml of rat [^{3}H]DNA in a total volume of 1.6 ml of 0.04 M phosphate (pH 6.8) and 0.1 mM dithiothreitol incubated 15 min at 0° and filtered over triplicate nitrocellulose filters. The data shown represent the average amount of DNA retained on the three filters. A background of less than 4% DNA retained on the filters in the absence of any NHC proteins has been subtracted.

Table I: Retention of NHC Proteins on Membrane Filters.[a]

Protein in Sample (μg)	Protein on Filter (μg)	Protein in Filtrate (μg)	% Protein Recovered
3.2	2.2 ± 0.2		68.7
6.5	4.2 ± 0.8		65.0
13.0	9.2 ± 1.9		70.8
19.5	12.5 ± 2.5		64.0
26.0	15.6 ± 3.1		60.0
32.0	18.6 ± 3.7		57.0
48.7	29.9 ± 5.9	3.2 ± 0.6	63.0
65.0	29.5 ± 6.1	7.8 ± 1.5	57.5

[a] Total NHC proteins were diluted in 0.04 M phosphate buffer (pH 7.1) and passed through the membrane filter. After filtration, 10% Cl_3CCOOH was passed over the membrane filter. Protein on the filter and the filtrate protein was determined by the Kuno and Kihara technique. Protein concentrations were determined by standard bovine serum albumin solution.

Results

DNA Binding Characteristics of the NHC Proteins. Rat liver NHC proteins may be separated into fractions based on their affinity toward DNA immobilized on a tandem series of DNA cellular columns (*E. coli* DNA-cellulose followed by rat DNA-cellulose). Under low ionic strength conditions (110 mM), three fractions of NHC proteins consisted of a non-DNA binding fraction (36%), an *E. coli* DNA-binding fraction (40%), and a rat DNA binding fraction (4%) (van den Broek *et al.*, 1973). We have investigated the affinity of the two classes of DNA-binding NHC proteins for DNA by the membrane filter assay.

The saturation characteristics of the total rat liver NHC proteins and the two DNA-binding NHC protein fractions are shown in Figure 1. At a constant amount of DNA (1.0 μg/ml), the total NHC protein mixture and the *E. coli* DNA-binding NHC protein fraction retain 60–70% of whole rat DNA at saturation. The rat DNA-binding NHC proteins retain 35% of whole rat DNA while the nonbinding proteins retain little or no DNA on the filter.

Table II: Resaturation of Isolated Protein-Bound DNA.

	DNA (μg)	
[^{3}H]DNA Saturated by Total NHCP[a]	Retained 50 μg (68%)	Filtrate 15 μg (20%)
Resaturation[b]		
E. coli DNA binding NHC proteins	1.01 ± 0.05 μg	1.10 ± 0.1 μg
Rat DNA binding NHC proteins	0.90 ± 0.09 μg	

[a] A saturating amount (3.0 mg) of total rat liver NHC protein was mixed with ^{3}H-labeled Novikoff ascites DNA (75 μg) in 25 ml of 0.04 M phosphate buffer and 0.1 mM dithiothreitol (pH 6.8). Five 5-ml aliquots were filtered over five nitrocellulose filters. The retained DNA was eluted off by sodium dodecyl sulfate-NaCl extraction (Probst *et al.*, 1971) and the filtrate DNA was repurified by Pronase digestion and phenol extraction. [b] These fractions were resaturated with the respective protein fraction; 6 μg of DNA was saturated with a 20-fold excess of protein, and triplicate samples were filtered over the nitrocellulose filter. The amount of DNA retained is reported as an average of the triplicate samples.

The saturation profiles are a reflection of the retention efficiency of the filter assay (Riggs *et al.*, 1970) which is dependent on several factors. First, the filter assay retains protein-bound DNA due to the retention of proteins on the filters (Hinkle and Chamberlain, 1972). A decrease in the retention of protein–DNA complexes may be a reflection of protein saturation of the membrane filters. To avoid such possibilities, the protein levels were kept below the protein saturation level of the nitrocellulose filter. Table I indicates the retention of protein on the filters (24 mm in diameter) is linear up to 32 μg of protein/sample. Protein samples above 32 μg of proteins per filter were not quantitatively retained. We have confined our observations to assays which contain less than 30 μg of protein/filter assay.

Second, the NHC protein fractions consist of a heterogeneous group of polypeptide components. The retention plateaus may be due to stability of the protein–DNA complexes or to a limited number of protein-binding sites on the DNA. Control experiments were run in which the protein–DNA complexes were diluted 4- to 18-fold immediately before filtration and the filtered complexes were washed with increasing wash buffer. The saturation plateaus were not altered indicating the stability of the complexes was not affected by the filtering process. As shown in Table II, whole rat DNA sheared to 350 base pair length was saturated with rat liver NHC proteins and separated into a fraction (68% of the total DNA) retained on the nitrocellulose filter and a fraction (20%) which passes through the filter. The 12% loss of DNA may be attributed to the purification of these two fractions. Resaturation of 2 μg of the filter-bound DNA with the *E. coli* DNA binding NHC proteins or the rat DNA-binding NHC proteins retained 50 and 45%, respectively. The DNA in the filtrate could not be resaturated by the rat DNA-binding NHC proteins whereas the *E. coli* DNA-binding NHC proteins retained 53% of this fraction. These results indicate that the *E. coli* DNA-binding NHC proteins retain a fraction of any DNA available to them and thus appear to be nonselective DNA-binding proteins. The rat DNA-binding NHC proteins appear to be more se-

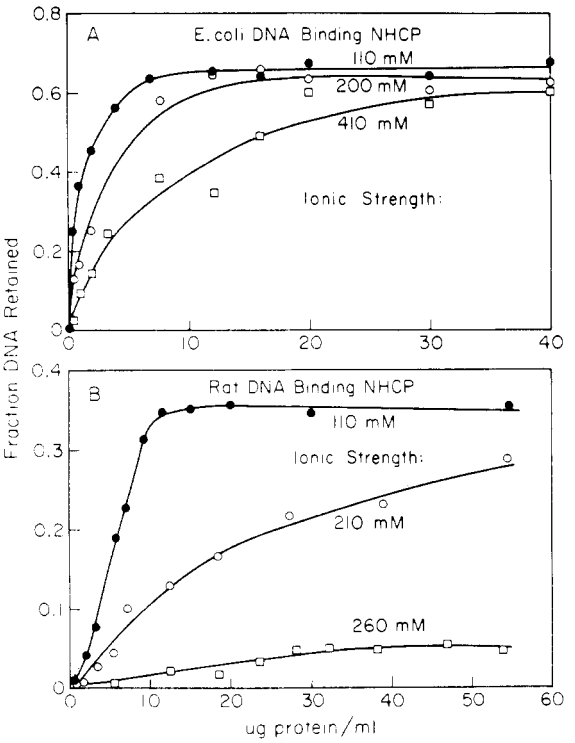

FIGURE 2: Ionic strength effect on the saturation of rat DNA. *E. coli* DNA binding NHC proteins and rat DNA binding NHC proteins were used to saturate 1 μg of labeled rat DNA in 1-ml assay mixtures. Triplicate samples were taken after 15-min incubation at 0°. Ionic strengths were increased with sodium chloride in 0.04 M phosphate buffer and 0.1 mM dithiothreitol (pH 6.8). The ionic strength levels are: 0.0 M NaCl; 110 mM; 0.15 M-260 mM, and 0.30 M NaCl-410 mM.

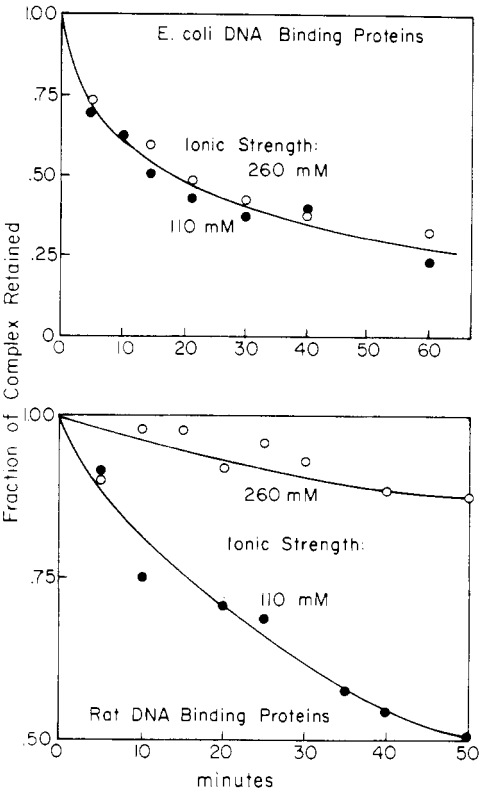

FIGURE 3: Dissociation of DNA-protein complexes. The dissociation of the *E. coli* DNA binding NHC protein-DNA complexes or the rat DNA binding NHC protein-DNA complexes were followed at 0.0 M NaCl (○) and 0.15 M NaCl (●). Limited protein was added to a constant amount of labeled rat DNA and allowed to reach equilibrium for 15 min at 0°. At zero time, a 20-fold excess of unlabeled rat DNA was added and triplicate samples were taken at each time point.

lective for the DNA which is bound and thus may interact with specific sites on the rat genome.

Characteristics of the Protein-DNA Complexes. As indicated above the degree of retention of the DNA-protein complexes may reflect a function of the specificity in the initial interaction between the DNA and the DNA-binding NHC proteins and the stability of the complex formed. Therefore, some of the characteristics of the DNA-protein interactions were investigated.

At 0, 22, or 37° there is no change in the saturation level of rat DNA by the total or the two DNA-binding NHC protein fractions. Protease activity, contained in the chromosomal protein mix (Garrels *et al.*, 1972; Chong *et al.*, 1974), was minimized by performing all experiments at 0°. Association occurred so rapidly that the rates of association could not be determined under these conditions.

The effect of increasing ionic strength (increasing concentrations of NaCl in the present of 0.04 M phosphate buffer) on the saturation profile of DNA binding to the several classes of NHC proteins is shown in Figure 2. The homologous, *i.e.*, rat, DNA-binding NHC proteins exhibit a strong dependence of binding to DNA upon the ionic strength of the medium. The heterologous, that is *E. coli* DNA binding NHC proteins, exhibit a constant level of saturation of DNA. The retention efficiency of the nitrocellulose filter is not affected by the ionic strength of the medium since increased washing of the filtered complexes or dilution of the sample before filtration does not affect the shape or plateau of the saturation curves. Rates of dissociation of the previously formed complex between the *E. coli* DNA-binding NHC proteins and the rat DNA-binding NHC proteins were measured by adding a 20-fold excess of unlabeled, sheared rat DNA to a limiting protein-[^{3}H]DNA mixture after a 15-min incubation (Figure 3). The amount of radioactive DNA retained on the filter is a direct measure of the concentration of undissociated complexes. Both complexes showed a substantial dissociation (>50%) after 60 min at low ionic strength (110 mM). At 260 mM ionic strength (0.04 M phosphate buffer, 110 mM ionic strength, 50 mM ionic strength, 0.15 M NaCl), the rat DNA-binding NHC protein-DNA complex is stable over 60 min whereas the *E. coli* DNA-binding protein complex is still unstable.

These data characterize the two fractions of NHC proteins as exhibiting different responses to ionic strength. The *E. coli* DNA-binding NHC proteins are substantially independent of over ionic strength but may be nonspecific in their DNA interaction. The rat DNA-binding NHC proteins include a group of complexes which are more stable at higher ionic strength.

Figure 4 demonstrates the specificity of the two DNA-binding fractions of the NHC proteins. Equilibrium competition studies were done at low ionic strength between labeled rat DNA and unlabeled rat and *E. coli* DNA as described. The *E. coli* DNA-binding NHC proteins show an equal affinity to either the *E. coli* DNA or the rat DNA. The rat DNA-binding NHC proteins show a substantial preference for rat DNA over *E. coli* DNA. The difference in the saturation profiles is apparently due to the limited number of sites on the rat genome for the rat DNA-binding NHC proteins or due to the fractionation of the DNA-bind-

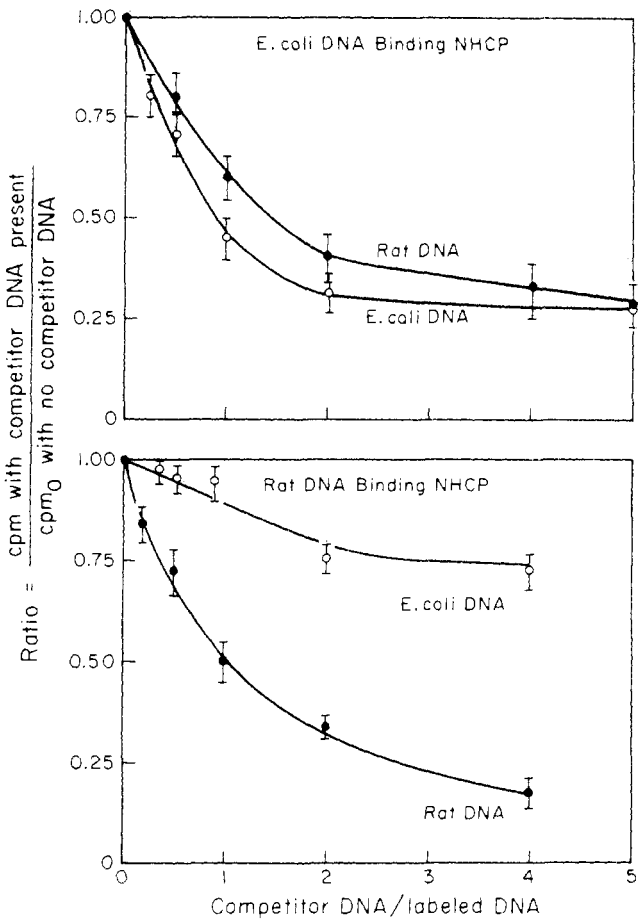

FIGURE 4: Competition studies. A limiting amount of *E. coli* DNA binding or rat DNA binding NHC proteins were mixed with a constant amount of labeled rat DNA and increasing amounts of unlabeled competitor DNA. The results are reported as the ratio of cpm at increasing competitive DNA/cpm without competitor DNA present.

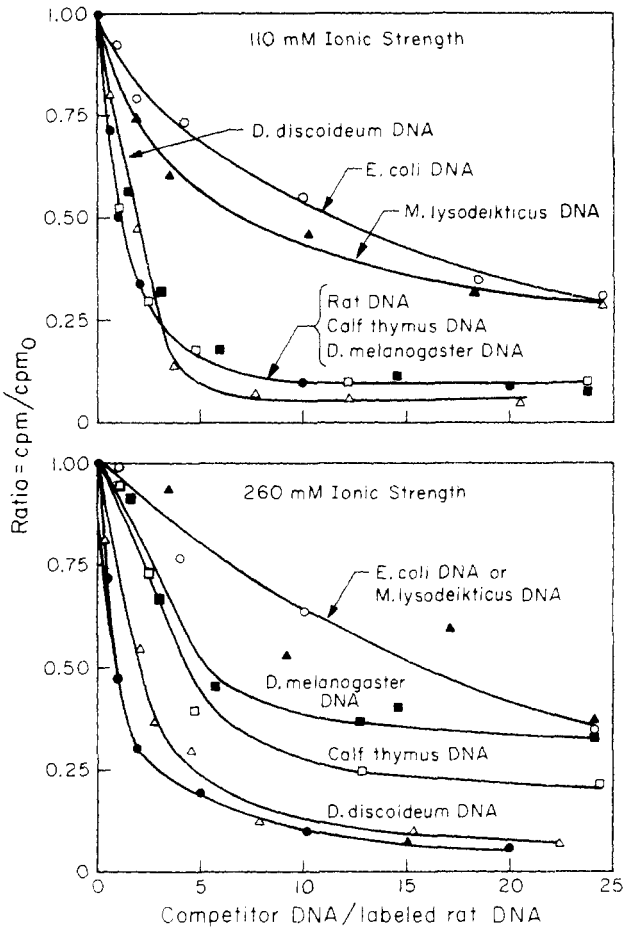

FIGURE 5: Competition of the rat DNA binding NHC proteins. Competitions as described in the Methods were run at 110 or 260 mM ionic strength. The competitors were rat (●), *E. coli* (○), *D. melanogaster* (□), *D. discoideum* (△), calf thymus (□), and *M. lysodeckticus* (▲).

ing NHC proteins which have a high affinity for nonspecific DNA interactions.

Specificity of Rat Liver NHC Protein-DNA Interaction. If the rat DNA-binding NHC proteins have a limited number of binding sites available in the rat genome, equilibrium competition studies should indicate a high specificity toward rat DNA. Figure 5 and Table III indicate at low ionic strength (110 mM-0.04 M phosphate buffer and 0.1 mM dithiothreitol (pH 6.8)) there is little preference between rat DNA and various other eukaryotic DNAs. Increased ionic strength decreases the number of interactions with the rat genome. Competitions indicate the rat DNA-binding NHC protein-DNA interaction also has an increased specificity for rat DNA. Competition by *D. melanogaster* DNA or calf thymus DNA had an increased ratio of competitor DNA/labeled rat DNA (for 50% competition) of 4.0- and 6.0-fold, respectively (Table III). However, *D. discoideum* DNA shows a constant ratio of 2.0 at low and high ionic strength levels. The 22 mol % guanine + cytosine base composition of *D. discoideum* suggests the competition effect may be base composition correlated. However, *D. melanogaster*, calf thymus, and rat liver DNAs all have similar base compositions (41 mol % guanine + cytosine) but have different competition ratios at the higher ionic strength. Our conclusion is protein-DNA interaction is sensitive to base composition, preferring adenine-thymine over guanosine-cytosine base pairs. Within the narrow range of the base composition of rat DNA (41 mol % G-C), the organization of the base sequences becomes important in protein-DNA interactions.

Table III: Competition Values of Various DNAs.[a]

DNA	Ratio Competitor DNA/Rat DNA for 50% Competition: 100 mM Ionic Strength	260 mM Ionic Strength	Mole % G-C
Rat	1.0	1.0	42.2
D. discoideum	2.0	2.0	22.0
D. melanogaster	1.0	4.0	40.0
Calf thymus	1.0	6.0	42.3
E. coli	11.5	16.75	50.6
M. lysodeckticus	7.5	16.75	70.0

[a] All DNAs were sheared to 350 base pairs. The assay contained 1 μg of ^{3}H-rat DNA and limiting NHC proteins sufficient to retain the DNA to 50% its saturation level. Competition ratios are reported as a fraction of competitor DNA/labeled DNA which reduces the retained complexes 50% from the control (no competitor DNA added).

Under certain conditions of reassociation, the rat genome contains 70% single copy sequences, 20% middle repetitive

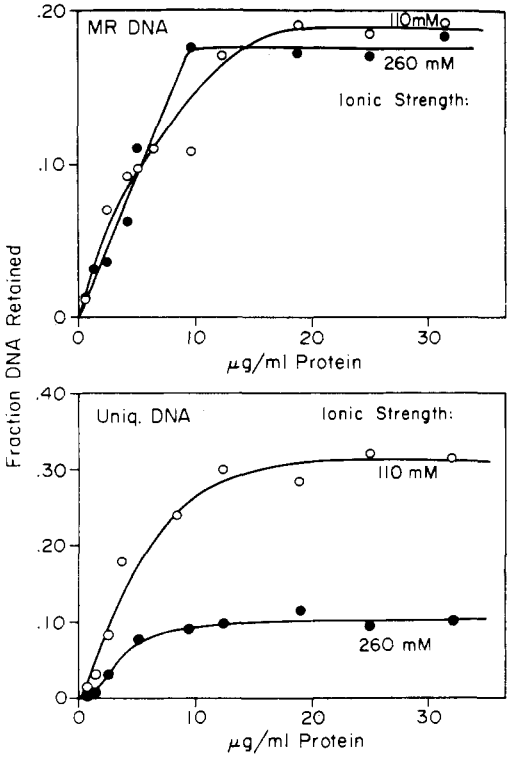

FIGURE 6: Saturation of the DNA components of the rat genome. Constant amounts of middle repetitive or single copy DNA (1 μg/ml) was saturated with increasing amounts of the rat DNA binding NHC proteins. The assays were run as described in Figure 2. Saturation characteristics were determined at low (110 mM–0.04 mPB) and high (260 mM–0.15 M NaCl, 0.04 mPB) ionic strength.

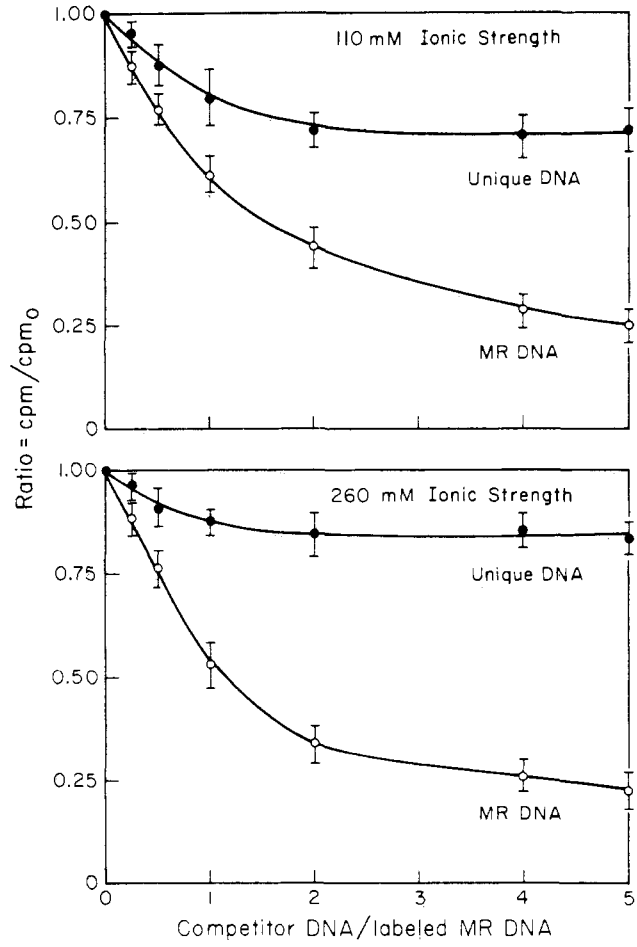

FIGURE 7: Competitions between DNA components. Competitions as described in Figure 4 and Methods were run between labeled middle repetitive DNA and unlabeled components of the rat genome. The competitions were carried out at low and high ionic strength.

sequences, and 10% highly repetitive DNA (Holmes and Bonner, 1974). To investigate the effect the repetition frequency of certain DNA sequences may have on the protein–DNA interactions, the individual components of the rat genome were separated and treated with S1 nuclease as described.

The reassociation kinetics of the isolated middle repetitive component show a half C_0t pure of 1.1 with an RMS of 0.0536. The single copy component half C_0t^{pure} was 1.6×10^3 with an RMS of 0.0473. The T_m characteristics in 0.12 M phosphate buffer of our middle repetitive and single copy DNA fractions were 76 and 81°, respectively, compared to 84.5° for native DNA indicating some mismatching is present (Ullman and McCarthy, 1973a,b). The recovery of 95% of the hyperchromicity demonstrates the double-stranded nature of our DNA complexes. But, it does not exclude that some single-stranded regions are still present.

The rat DNA-binding nonhistone chromosomal proteins saturate both middle repetitive DNA and single copy DNA. The level of saturation of middle repetitive DNA sequences by homologous DNA-binding proteins is independent of ionic strength, as shown in Figure 6. Binding of single copy sequences by the same proteins is not independent of ionic strength, and is almost eliminated at 260 mM ionic strength. Therefore, the middle repetitive DNA sequences of the rat genome bind rat DNA-binding proteins with greater stability at physiological ionic strength than do the single copy sequences.

By calculation, the total rat genome bound by the rat DNA-binding NHC proteins at low ionic strength is 25% ($0.19 \times 0.2 + 0.7 \times 0.32$) where the experimental value is 35% (Figure 2). At high ionic strength (260 mM), the observed DNA retention is 5% while the calculated retention is 10% ($0.17 \times 0.2 + 0.7 \times 0.1$). These calculations suggest that 260 mM ionic strength conditions reduce the protein–DNA interactions of the unique DNA sequences in the rat genome but that the middle repetitive DNA–protein interactions are stable under these conditions. Figure 7 shows the competition between labeled middle repetitive DNA and single copy DNA at high and low ionic strength. The competition by the middle repetitive DNA is very efficient under both conditions. While the single copy DNA sequences are inefficient competitors (plateau at 25% competition) under low ionic strength conditions, at high ionic strength they become even less efficient and reach a competition plateau of 15% at a fivefold excess of single copy DNA. Competition with labeled single copy DNA could not be run at high ionic strength since the complex retention is negligible. At low ionic strength, the competitions were highly efficient for both middle repetitive and single copy DNA (data not shown). Under our assay conditions, the rat DNA-binding NHC proteins show a substantial preference for middle repetitive DNA sequences.

Discussion

Eukaryotic organisms are characterized by their large genomes with fractions of DNA of a higher complexity than observed in prokaryotes (Britten and Kohne, 1968). Most eukaryotic genomes are also characterized by chro-

mosomal proteins associated with the DNA which are thought to be involved in the structure and function of the particular genome (Bonner *et al.*, 1968). A prerequisite characteristic of such proteins would be the capacity to interact specifically with their homologous genome. Recent investigations of the chromosomal proteins of the rat liver chromosomes has isolated 1.0–1.5% of the total nuclear proteins which bind rat DNA with some specificity (Kleinsmith *et al.*, 1970; Kleinsmith, 1973; Teng *et al.*, 1971; Patel and Thomas, 1973). In this laboratory, we have developed a technique where 4% of the total rat liver NHC proteins may be isolated which have a high preference for interaction with rat DNA (van den Broek *et al.*, 1973). We have extended these studies by employing a nitrocellulose filter assay procedure to monitor protein–DNA interaction (Riggs *et al.*, 1970; Wilcox *et al.*, 1974).

A quantitative analysis of the NHC protein–DNA interactions has not yet been developed due to several difficulties. First, it is apparent that we have investigated a very specific group of DNA binding NHC proteins. Our binding assays indicate that the ionic medium can influence the specificity of DNA–protein interaction by decreasing the nonspecific interactions of the rat DNA binding NHC proteins at higher ionic strength.

This is in contrast with the well-defined *lac* repressor–operator interaction where increasing ionic strength decreases the binding constants for both the operator and nonoperator interaction. The conclusions of the repressor–operator investigations were that the repressor possesses a general weak affinity for DNA and that conditions could not be found which maximizes specific DNA interaction (Lin and Riggs, 1972). Therefore, the *E. coli* DNA binding NHC proteins may include proteins which bind specifically to rat DNA but that under the present conditions, specific interaction cannot be detected due to the weak nonspecific affinity of these proteins.

Second, the technique is limited as to determination of the number of protein binding sites available per rat genome. An accurate measure of the amount of DNA retained by the NHC proteins on the nitrocellulose filters is dependent on the size of the DNA employed in the assay mix. The 350 base pair size of DNA was used due to the isolation procedure of the middle repetitive DNA sequences. However, the actual amount of DNA bound by the proteins has not been determined. Information on the characteristics of the DNA sites bound by the NHC proteins is now under investigation.

Third, the filter assay measures only the DNA to which one or more protein molecules are bound. In systems where a single DNA binding protein has been isolated and its DNA binding interactions investigated, the DNA binding protein consists of oligomers as the *lac* repressor (Riggs *et al.*, 1970) or the ara C protein (Wilcox *et al.*, 1974) or a subunit interaction which imparts a more stringent DNA site selection on the core protein (Hinkle and Chamberlain, 1972; Chadwick *et al.*, 1970).

In these studies, an attempt was made to investigate the optimum conditions for DNA binding interactions. However, an analysis of the NHC protein fraction and the involvement of subunit interaction must be determined before quantitative analysis is possible.

Within these limitations, the NHC protein–DNA interactions can be discussed. The *E. coli* DNA binding NHC proteins (30% of the total NHC proteins) have a high affinity for DNA in general. The DNA interactions appear to be independent of ionic strength. However, the specificity of these proteins with rat DNA at increasing ionic strength has not been investigated.

The rat DNA binding NHC proteins show substantially different DNA specificity. There are a limited number of sites available on the DNA at low ionic strength conditions for the rat DNA binding NHC proteins. These sites are completely saturated and retained by the NHC proteins on nitrocellulose filters; thus a measure of the amount of DNA (depending on size) retained by the proteins can be made. Two classes of sites for the rat DNA binding NHC proteins can be observed, although each class may be heterogeneous with respect to binding constants and numbers. One class of DNA binding sites are insensitive to increases of ionic strength up to physiological levels (260 mM). This class of sites constitute 17% of the middle repetitive DNA sequences and appear to represent 80% of the interactions between the rat DNA binding NHC protein and the total rat genome at physiological ionic strength (260 nM). The second class of rat DNA binding sites are lost at increased ionic strength (260 mM ionic strength). These sites are nonspecific for middle repetitive DNA, single copy DNA, and other eukaryotic DNAs at low ionic strength. The nonspecific interactions constitute 85% of the rat DNA binding NHC protein interaction with the total rat genome at low ionic strength. At high ionic strength, the second class of the interactions constitute approximately 20% of the protein–DNA interactions. We have, therefore, a 25-fold enriched fraction of NHC proteins from rat liver which preferentially binds middle repetitive rat DNA.

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On the Stimulation of Viral DNA Polymerase Activity by Nonionic Detergent†

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ABSTRACT: Nonionic detergents stimulate purified RNA-directed DNA polymerase (reverse transcriptase) activity from various RNA tumor viruses ranging from avian to primate species. The stimulatory effect of the nonionic detergent is dependent on the type and amount of template-primer. The greatest stimulation is obtained when high concentrations of $(dT)_{12-18} \cdot (rA)_n$ or activated salmon sperm DNA are used as template-primers. Little stimulation is obtained with viral 70S RNA or with $(dT)_{12-18} \cdot (dA)_n$. The detergent stimulation appears to be specific for viral reverse transcriptase since this effect is not observed with purified bacterial DNA polymerase or with three known mammalian cellular DNA polymerases. This finding may, therefore, be a useful additional criterion for distinguishing viral reverse transcriptase isolated from cells from other cellular DNA polymerases. Nonionic detergent also has a stabilizing effect on viral DNA polymerase against thermal inactivation. This stabilizing effect is further enhanced by the presence of template-primer.

Reverse transcriptase of RNA tumor viruses is located in the cores of the virus structure (Gerwin *et al.,* 1970; Coffin and Temin, 1971). In order to detect the maximum activity of this enzyme, virus particles are usually disrupted with a predetermined optimal concentration of nonionic detergent (Baltimore, 1970; Temin and Mizutani, 1970; Scolnick *et al.,* 1970; Bishop *et al.,* 1971). Recently, nonionic detergents were shown to stimulate $(dT)_n$ synthesis with purified murine leukemia sarcoma virus reverse transcriptase using $(dT)_{12-18} \cdot (rA)_n$ as template-primer (Thompson *et al.,* 1972; Wu *et al.,* 1973; Wu and Gallo, 1974). Therefore, nonionic detergents are used not only to disrupt virus particles and solubilize viral reverse transcriptase but also to enhance the activity of the viral enzyme. The purpose of this study is to further elucidate the mechanisms of this enhancement of DNA polymerase activities by nonionic detergent.

Our results indicate that the stimulatory effect of nonionic detergents is dependent on the type and amount of template-primer. The largest stimulation is obtained when high concentrations of $(dT)_{12-18} \cdot (rA)_n$ or activated salmon sperm DNA are used as template-primers. Very little stimulation is observed with viral 70S RNA or with $(dT)_{12-18} \cdot (dA)_n$. The stimulation appears to be specific for viral reverse transcriptase since there is no effect on bacterial and mammalian cellular DNA polymerases. Studies on thermal inactivation of the enzyme show that nonionic detergents slightly stabilize the activity of the enzyme. The enzyme stability is further increased by the presence of template-primer.

Materials and Methods

Nonionic Detergents and Template-Primers. The nonionic detergents used in this study were obtained from the

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