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Phosphorylation and DNA Binding of Nuclear Rat Liver Proteins Soluble at Low Ionic Strength†

Archie W. Prestayko,* Paula M. Crane, and Harris Busch

ABSTRACT: Proteins were extracted from isolated rat liver nuclei with 0.15 M NaCl and 0.35 M NaCl at pH 8.0. The number of phosphoproteins in these extracts was determined by labeling with ^{32}P and autoradiography after two-dimensional gel electrophoresis. Two proteins, B22p and B24p, contained small amounts of ^{32}P and sedimented with the 30S nuclear informofer particle. With the exception of two phosphoproteins, CB and CN′, all of the phosphoproteins found in the 0.35 M NaCl extract of nuclei were also present in the 0.15 M NaCl extract. Approximately 20% of the 0.15 M NaCl soluble proteins bound to rat liver DNA in 0.05 M KCl–0.05 M Tris-HCl (pH 8). Of these proteins, 1–2% bound to DNA in 0.15 M KCl and were eluted with 2 M KCl. This DNA bound fraction which contained both phosphorylated and nonphosphorylated proteins was similar in both the 0.15 and 0.35 M NaCl extracts. However, two major proteins (C13 and C14) and three minor proteins (C15, C25, Cg′) were present only in the 0.15 M NaCl extract. The results of the present study show that there are marked similarities in the two-dimensional gel electrophoretic, phosphorylation, and DNA binding properties of rat liver nuclear proteins soluble in either 0.15 or 0.35 M NaCl.

Recently, studies have been made on nuclear proteins and their role in chromatin structure and function, particularly proteins "loosely" bound to chromatin that are extracted at low ionic strength (Patel, 1972; Comings and Tack, 1973; Holoubek and Fujitani, 1973; Kostraba and Wang, 1973; Kostraba et al., 1975). The involvement of "tightly" bound proteins in chromatin structure and function (Paul and Gilmour, 1966, 1968; Spelsberg and Hnilica, 1969; Spelsberg et al., 1971; Kostraba and Wang, 1972) has been recently reviewed (Olson and Busch, 1974; Stein et al., 1974).

Early studies on proteins soluble in Tris-saline buffers (Gurdon and Brown, 1965; Goldstein and Prescott, 1967, 1968; Gurdon, 1970; Goldstein, 1974) in *Amoeba* and *Xenopus* demonstrated that a number of nuclear proteins diffuse readily across the nuclear membrane; they have been referred to as cytonucleoplasmic shuttling proteins. More

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recent studies have suggested that cytoplasmic and nuclear proteins soluble in 0.15–0.35 M NaCl that bind to DNA (Kostraba et al., 1975; Salas and Green, 1971; Tsai and Green, 1973; Vaughan and Comings, 1973; Johnson et al., 1975) may be involved in regulation of transcription (Van den Broek et al., 1973; Sevall et al., 1975; Kleinsmith et al., 1966; Kleinsmith, 1973; Kostraba et al., 1975), possibly after phosphorylation.

Proteins that shuttle between nucleus and cytoplasm include hormone receptor proteins. These proteins have been shown to bind to chromatin and stimulate transcription of specific messenger RNA (Spelsberg et al., 1971; Rosen et al., 1974).

Materials and Methods

Protein Isolation and Electrophoresis. Rat liver sucrose nuclei were prepared by a modification (Busch and Smetana, 1970) of the Chauveau procedure (Chauveau et al., 1956). Proteins were extracted from nuclei according to the scheme in Figure 1. Nuclei were extracted twice with 10 volumes of 0.01 M Tris-HCl buffer (pH 7.0) containing 0.15 M NaCl, 0.001 M $MgCl_2$, and 0.1 mM phenylmethanesulfonyl fluoride ($PhCH_2SO_2F$)[1] (Ballal et al., 1975) followed by four extractions with the same buffer at pH 8.0 (Samarina et al., 1968). Following these extractions, nuclei were further extracted twice with 10 volumes of 0.35 M NaCl in 0.01 M Tris-HCl buffer at pH 8.0. After each extraction the samples were centrifuged at 8000*g* for 10 min. The supernatants were centrifuged at 142 000*g* for 4 h in a Beckman No. 35 rotor to remove dense RNP particles and were concentrated in an Amicon apparatus on a UM-2 filter to a concentration less than 1 mg/ml. The Tris-saline extract at pH 8.0 was centrifuged on a 10–50% sucrose gradient at 82 500*g* in a Beckman SW 27 rotor. Fractions from the sucrose gradient and aliquots of the original samples were mixed with equal volumes of 8 M urea and 4 M LiCl and stirred at 4° for 16 h. Precipitated nucleic acids were removed by centrifugation and the supernatant proteins were dialyzed against 0.9 N acetic acid containing 9 M urea and 1% β-mercaptoethanol for two-dimensional gel electrophoresis (Orrick et al., 1973)[2] or against 0.05 M Tris-HCl (pH 8.0) containing 0.05 M KCl for DNA binding studies.

Preparation and Extraction of Chromatin. Chromatin was prepared from nuclei by two washes with 0.075 M NaCl, 0.025 M NaEDTA (pH 8.0), and 0.0001 M $PhCH_2SO_2F$ and three washes of the residue with 0.01 M Tris (pH 8.0) and 0.0001 M $PhCH_2SO_2F$. Samples were centrifuged at 15 000*g* for 15 min after each extraction. The pellet was extracted twice with 0.01 M Tris-HCl (pH 8.0) containing 0.35 M NaCl and 0.0001 M $PhCH_2SO_2F$ and centrifuged at 30 000*g* for 20 min. The supernatants were concentrated and treated as described above.

Phosphoprotein Labeling. Male Sprague-Dawley rats were injected i.p. with 20 mCi of [^{32}P]inorganic phosphate (Union Carbide Corp., Tuxedo, N.Y.) 2 or 4 h prior to sacrifice. Liver nuclei were prepared as described above. Proteins were extracted with 2 M LiCl–4 M urea containing 0.001 M potassium fluoride to prevent phosphatase activity

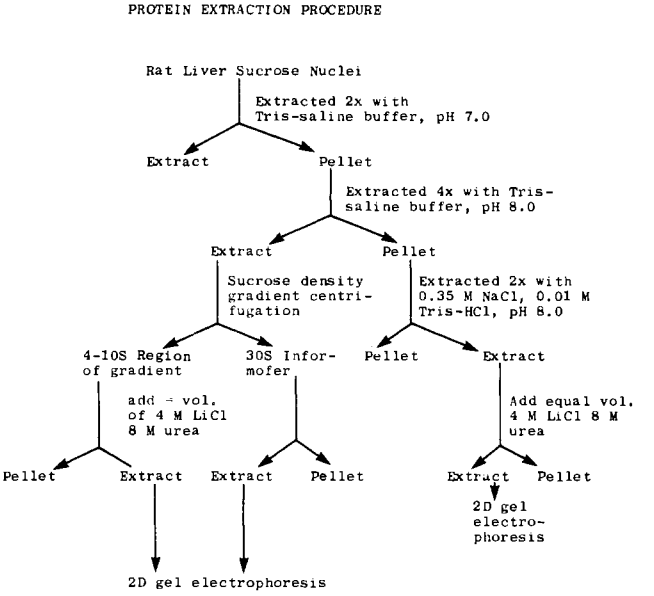

FIGURE 1: Procedure for extraction of proteins from rat liver sucrose nuclei.

(Schmidt and Laskowski, 1961). Following two-dimensional gel electrophoresis, the gels were dried under vacuum and subjected to autoradiography (Olson et al., 1974b).[3]

DNA–Polyacrylamide Column Chromatography. DNA–polyacrylamide column chromatography was performed as previously described (Cavalieri and Carroll, 1970; Vaughan and Comings, 1973). Rat liver DNA was prepared by a modified Marmur procedure (Marmur, 1961; Sitz et al., 1973). DNA–polyacrylamide was prepared by mixing 12.5 ml of a solution containing 0.05 M Tris-HCl (pH 7.8), 9.7% acrylamide, 0.3% bisacrylamide, 2.5–12.5 mg of DNA, and 0.1 ml of tetramethylethylenediamine with 12.5 ml of 0.05 M Tris-HCl buffer (pH 7.8) containing 10% (w/w) agarose at 50°. The temperature immediately dropped to 37° at which time the DNA showed less than 1% hyperchromicity. After the mixture was stirred and cooled to 33°, 0.016 g of ammonium persulfate was added in 0.25 ml of H_2O. After 1 h of polymerization the gel was cut into pieces and pressed through a stainless steel wire mesh (no. 80), washed six times with 5 volumes of 0.05 M Tris-HCl buffer (pH 7.8), made into a slurry, and packed in a 1 × 30 cm column. The eluent was pumped from the bottom at a flow rate of 6–10 ml/h. Protein samples of 10–15 mg were applied to the column in 0.05 M Tris-HCl (pH 8.0) containing 0.05 M KCl and eluted with 0.15 and 2.0 M KCl. Column fractions were dialyzed against 0.9 N acetic acid and lyophilized and the proteins were analyzed by two-dimensional gel electrophoresis.

Results

0.15 M NaCl Soluble Nuclear Proteins. The two-dimensional gel electrophoresis pattern of the ^{32}P-labeled total Tris-saline soluble protein fraction is shown in Figure 2. Since ribosomal or other proteins of low molecular weight and high electrophoretic mobility (Prestayko et al., 1974a) are absent from this nuclear extract, the proteins were sepa-

[1] Abbreviations used are: $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride; RNP, ribonucleoprotein.

[2] The one-dimensional gel system used was the first dimension gel electrophoresis condition of the two-dimensional electrophoresis procedure (Orrick et al., 1973). Gels were sliced in 1-mm slices and counted in a liquid scintillation counter.

[3] Previous studies in this laboratory have established that the phosphorylation of nuclear proteins as analyzed by the two-dimensional gel electrophoresis method is due to incorporation of radioactive phosphate mainly into serine residues (Olson et al., 1974a; Prestayko et al., 1974b).

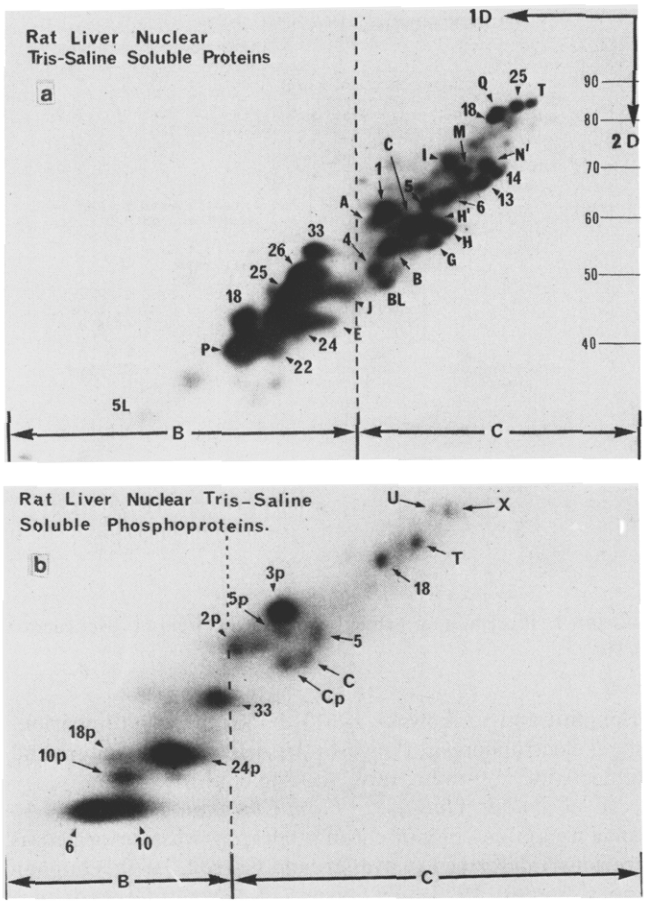

FIGURE 2: Two-dimensional gel electrophoresis of proteins of unfractionated Tris-saline extracts of rat liver sucrose nuclei. (a) Proteins were stained with 0.2% Coomassie Brilliant Blue in an 8% acetic acid and 40% methanol solution for 4–5 h. Arrows indicate the directions of the first (6% acrylamide) and second dimension (8% acrylamide) gel electrophoresis. The molecular weights of proteins migrating in the second sodium dodecyl sulfate dimension are expressed in 10^{-3} at the right. The gel was arbitrarily divided into B and C regions (Orrick et al., 1973) on the basis of mobility of spots B33 and B24. (b) Autoradiograph of the unfractionated Tris-saline extract in (a). Rats were labeled in vivo with ^{32}P for 2 h.

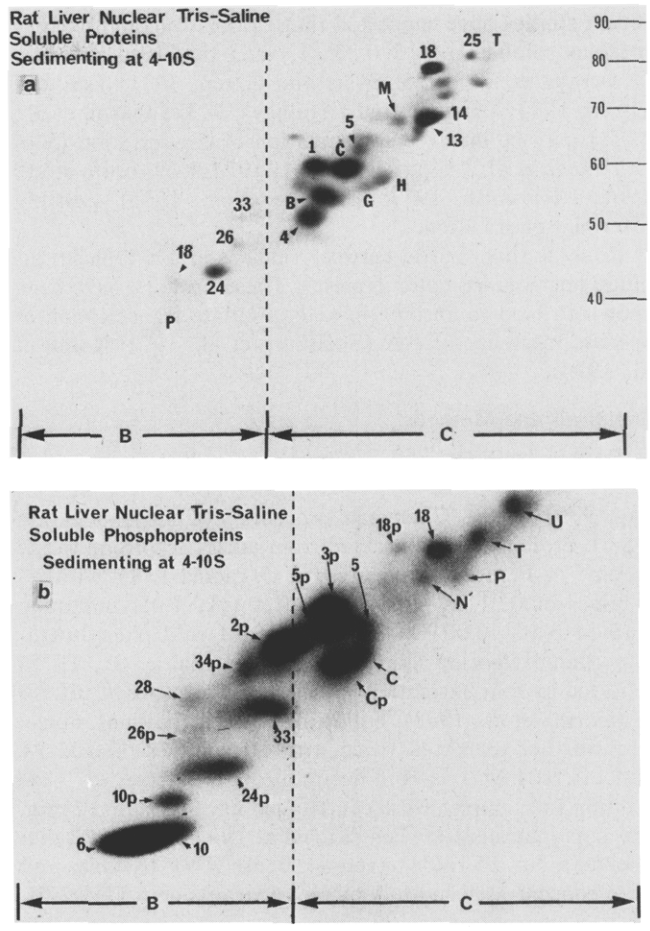

FIGURE 3: Two-dimensional gel electrophoresis of Tris-saline soluble nuclear proteins. (a) Stained pattern of proteins sedimenting at the 4–10S region of a sucrose gradient. Molecular weights are expressed in 10^{-3} at the right. (b) Autoradiograph of protein gel in Figure 3a.

rated on gels of low acrylamide concentration (6% in the first dimension and 8% in the second dimension) (Busch et al., 1974) to achieve better separation of the proteins of higher molecular weight (greater than 35 000). The stained pattern of the protein spots from the unfractionated Tris-saline extract and the autoradiograph of this pattern are shown in Figure 2a and b, respectively. Of approximately 30 major stained spots, 10 contain radioactive phosphate. The radioactive spots labeled as 10p, 18p, 24p, 2p, 5p, 3p, and Cp migrate very near to but do not coincide with the corresponding stained spot. Proteins B6 and B10 are also highly labeled (Figure 2b) proteins which are very faintly stained (Figure 2a). The densely stained spots (B22,24,25,26) which are the major proteins of the nuclear informofer particle contain very little label.

For further purification, the Tris-saline (pH 8.0) soluble fraction from rat liver sucrose nuclei was centrifuged on a 10–50% sucrose gradient. A slowly sedimenting fraction was present near the top of the gradient and a fraction was obtained at about 30 S corresponding to sedimenting nuclear informofer particles (Samarina et al., 1968). Figure 3a and b shows the stained and autoradiographic gel patterns, respectively, of the proteins sedimenting in the 4–10S region of the sucrose gradient. Most of the high molecular weight (C region) proteins of the whole Tris-saline extract (Figure 2a) are present in the 4–10S fraction of the gradient (Figure 3a) and the phosphoproteins in this region of the gels are similar. Highly labeled proteins B6 and B10 are present in the 4–10S fraction. Radioactive spots 2p, 3p, 5p, and Cp are more dense and spot 24p of the B region is less intense in Figure 3b than in Figure 2b.

Proteins of the nuclear 30S informofer particle were analyzed by both one-dimensional and two-dimensional gel electrophoresis. Figure 4 shows an absorbance and radioactivity profile of a stained acid–urea one-dimension gel of informofer proteins.[2] One major peak and two minor peaks of absorbance were detected in fractions 48–60. The ^{32}P counts coincided with the major peak and the faster migrating minor peak. Smaller peaks of absorbance were detected in the higher molecular weight region of the gel (fractions 20–40) but little radioactivity was present in these fractions. Figure 5a and b, which are different loads of the same protein fraction in Figure 4, show the stained protein spot patterns after two-dimensional gel electrophoresis. Proteins B22, 24, 25, and 26 are present in large amounts in the informofer particle in contrast to their low concentrations in the 4–10S region of the gradient (Figure 3a). Faint protein spots in the C region of the gel were visible when large sample loads were used (Figure 5b).

Figure 5c shows the autoradiograph of the gel in Figure 5b. Proteins of the informofer particles contained small

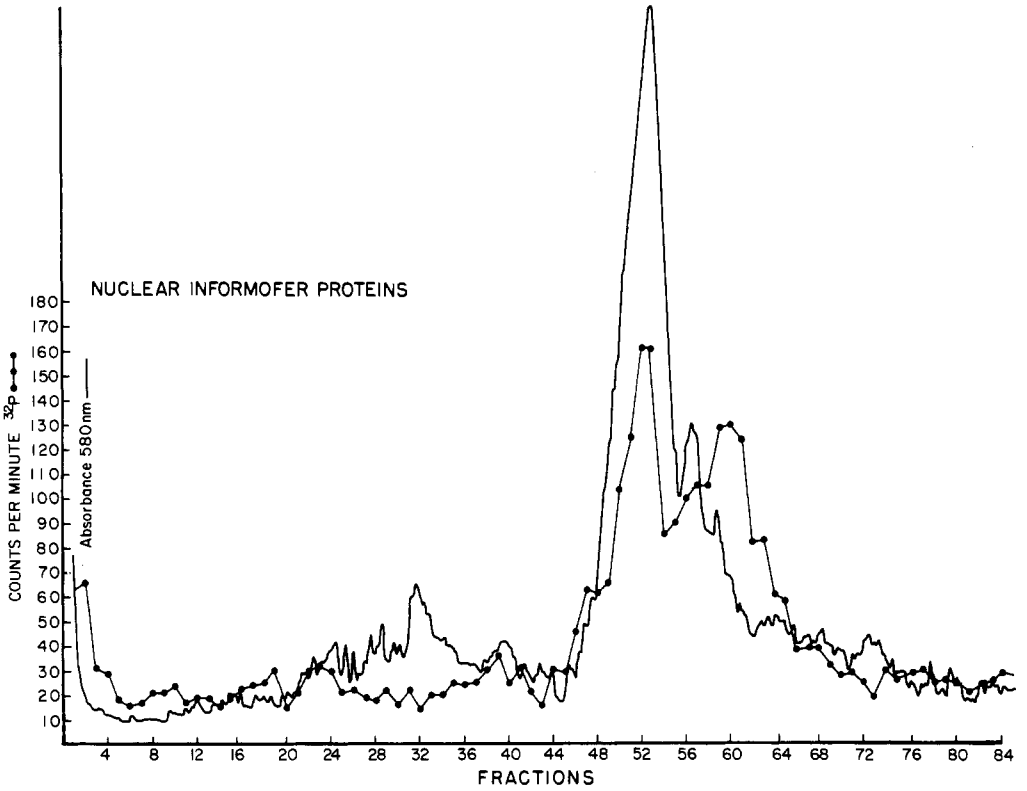

FIGURE 4: One-dimensional polyacrylamide gel electrophoresis in 0.9 N acetic acid and 4 M urea of ^{32}P-labeled rat liver nuclear informofer proteins. The gel was stained with Buffalo Black, scanned at 580 nm, and sliced into 1-mm slices. Slices were counted in a liquid scintillation counter. (—) Absorbance 580 nm; (●) counts per minute ^{32}P. Direction of protein mobility is from left to right.

amounts of ^{32}P radioactivity and were detected by autoradiography after 4 h of labeling in vivo and 18 to 21 days of exposure to autoradiography. These results show that most of the phosphoproteins in the nuclear Tris-saline extract are not in large ribonucleoprotein particles.

0.35 M NaCl Soluble Nuclear Proteins. Following the Tris-saline extractions at pH 8.0, nuclei were extracted twice with 0.35 M NaCl to determine whether phosphoproteins extracted with the higher ionic strength buffer were different from those extracted with Tris-saline. Figure 6a and 6b show the stained two-dimensional gel and autoradiographic pattern, respectively, of the 0.35 M NaCl soluble nuclear proteins. The 0.35 M NaCl extract contained a relatively higher proportion of proteins C5, CM, and C18 and a lesser proportion of proteins B33 and CB than the Tris-saline extract. Of the major radioactive protein spots (B33, C3p, and C18), protein spot C18 appeared to be more highly labeled with ^{32}P in the 0.35 M NaCl extract. In addition, protein spots CB and CN′ were labeled with ^{32}P in the 0.35 M NaCl extract but not in the Tris-saline extract. A comparison of relative staining and labeling intensities of the nuclear proteins is given in Table I.

To determine which of the 0.35 M NaCl soluble nuclear proteins are present in rat liver chromatin, rat liver nuclei were prepared by the citric acid procedure (Taylor et al., 1973) and chromatin was prepared as described in Materials and Methods. Figure 7 shows the two-dimensional gel electrophoresis pattern of the 0.35 M NaCl soluble protein of isolated rat liver chromatin. Although fewer protein spots were present in 0.35 M NaCl extracts of chromatin than in 0.35 M NaCl extracts of nuclei, many of the major protein spots had the same electrophoretic mobilities (C18, CN′, C6, C1, CA, B24, and BP). These results indicate similarities of proteins of the 0.35 M extracts of nuclei prepared by either the citric acid or sucrose methods. Many of these proteins are also major protein spots in the Tris-saline extracts (Figure 2a).

DNA Binding Proteins. To study DNA binding proteins of the 0.15 M NaCl and 0.35 M NaCl nuclear extracts, protein extracts were dialyzed against 0.05 M Tris-HCl (pH 8.0) containing 0.05 M KCl and chromatographed on rat liver DNA polyacrylamide columns. Approximately 80% of the Tris-saline extracted protein applied to the column did not bind to DNA in 0.05 M KCl. Of the proteins that bound to DNA, 98% were eluted with 0.15 M KCl and 1.5–2% were subsequently eluted with 2 M KCl. The stained gel electrophoresis patterns of the Tris-saline soluble nuclear proteins eluted from the DNA polyacrylamide column with 0.15 M KCl and 2 M KCl are shown in Figure 8a and b, respectively. Approximately 20 proteins are eluted with 0.15 M KCl (Figure 8a) and 8 major proteins are eluted with 2 M KCl (Figure 8b). Proteins CA, CC, CN′, and C15′ are present in both fractions; however, proteins C13, C14, Cg′, and C18 are major proteins eluted with 2 M KCl.

To determine whether phosphoproteins were bound to DNA, the stained gels were dried and subjected to autoradiography. Figure 9a and b represent ^{32}P-labeled proteins which were eluted from the DNA–polyacrylamide column with 0.15 and 2.0 KCl, respectively. Two major phosphoproteins (B33 and CN′) and one minor phosphoprotein (Cp) were eluted with 0.15 M KCl (Figure 9a) and five major phosphoproteins (B33, B34p, CN′, C18, and CT) were eluted with 2 M KCl. Of the total radioactive protein loaded on the DNA column, 87% of the radioactivity was eluted in the void fraction of the column, 7% was eluted in

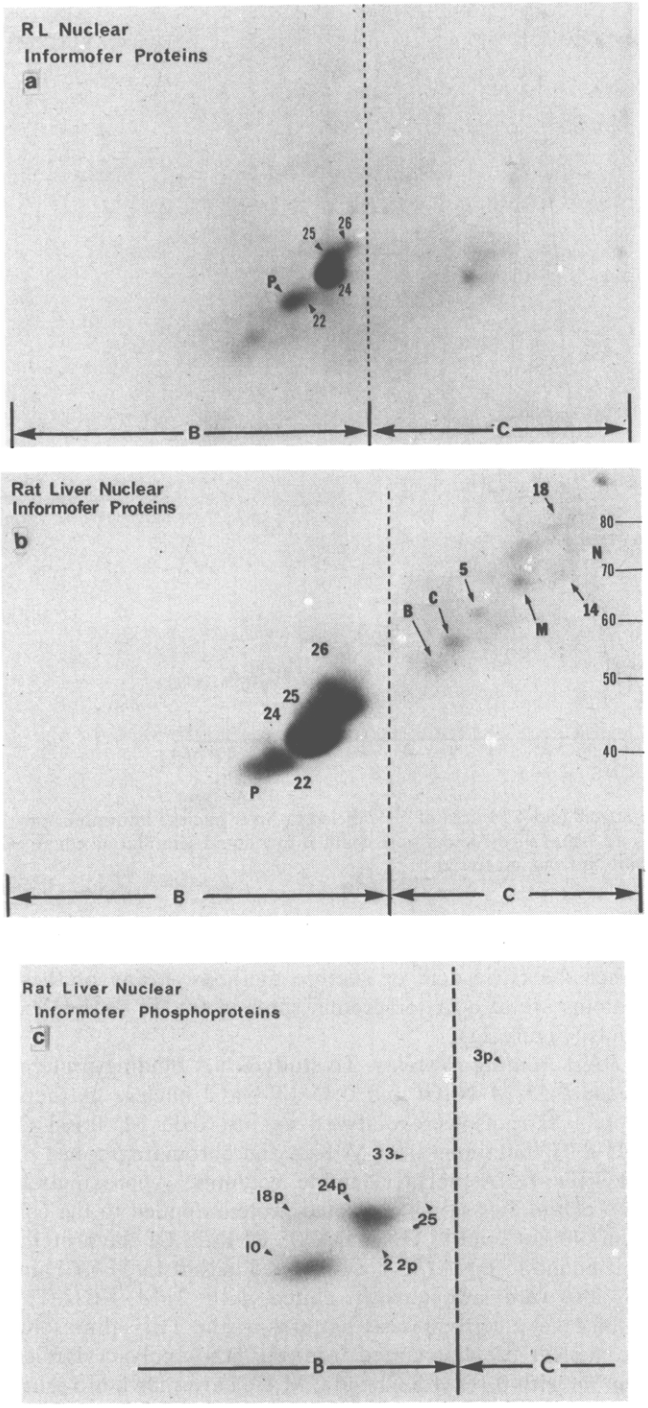

FIGURE 5: Two-dimensional gel electrophoresis of informofer proteins. (a) 50 μg of informofer proteins loaded on gel. (b) 250 μg of informofer proteins loaded on gel. Molecular weights are expressed in 10^{-3} at the right. (c) Autoradiograph of Figure 5b.

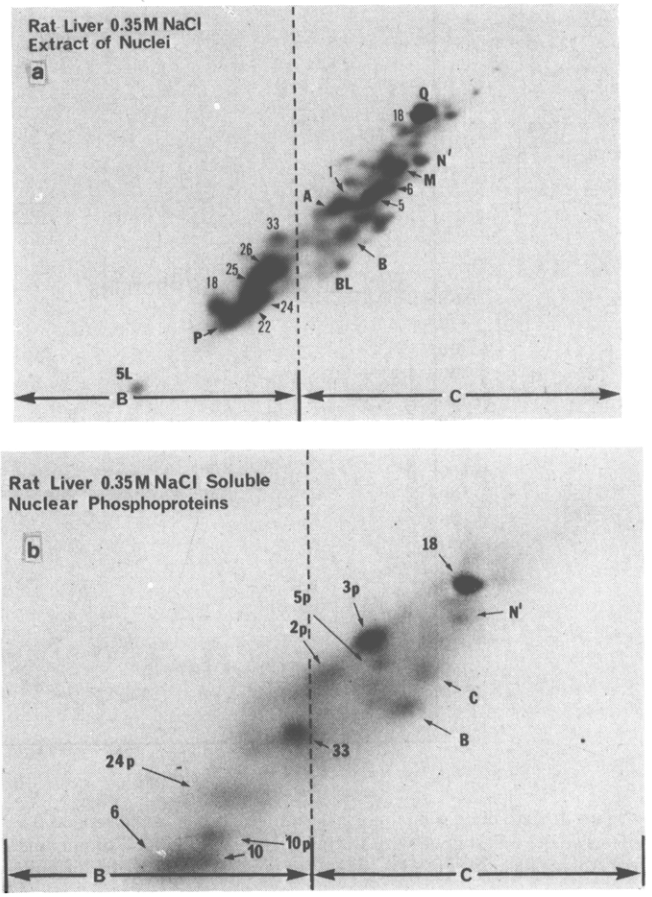

FIGURE 6: Two-dimensional gel electrophoresis of 0.35 M NaCl soluble rat liver nuclear proteins. (a) Proteins extracted from rat liver sucrose nuclei with 0.35 M NaCl after two extractions with Tris-saline buffer at pH 7.0 and four extractions at pH 8.0. (b) Autoradiograph of 0.35 M NaCl soluble proteins from sucrose nucleoli in Figure 6a.

the 0.15 M KCl fraction, and 5% was eluted in the 2 M KCl fraction. When proteins were chromatographed on polyacrylamide columns lacking DNA, no protein was bound to the column indicating that proteins that bind to the column bind to DNA.

To determine whether nuclear proteins that are extracted with 0.35 M NaCl bind more tightly or more specifically to rat liver DNA than 0.15 M NaCl soluble nuclear proteins, DNA binding studies were performed with the 0.35 M NaCl soluble nuclear protein fraction. To eliminate nonspecific binding of proteins to DNA, 0.35 M NaCl soluble proteins were first applied to an *Escherichia coli* DNA–polyacrylamide column in 0.15 M KCl–0.05 M Tris-HCl (pH 8.0). The unbound fraction was then applied to a rat liver DNA–polyacrylamide column in 0.15 M KCl and the bound protein fraction was eluted with 2 M KCl. A two-dimensional gel electrophoresis pattern of the 2 M KCl eluted proteins is shown in Figure 10. Two major proteins (CA and CC) and five minor proteins (A18, B33, CN′, C18, and CT) were eluted in this fraction. A comparison of DNA binding proteins of the 0.15 and 0.35 M NaCl extracts is shown in Table II. With the exception of protein A18, all of the DNA binding proteins present in the 0.35 M NaCl extract were present in the 0.15 M NaCl extract. Furthermore, the DNA binding fraction of the 0.15 M NaCl extract contained two phosphoproteins (B34p and C5) and five nonphosphorylated proteins (C13, 14, 15′, 25, g′) which were not present in the 0.35 M NaCl extract.

Discussion

Although protein phosphorylation has been studied in many cell components including nuclei, nucleoli, chromatin, ribosomes, and nucleolar preribosomal particles, the function of phosphoproteins in these cell constituents has not been precisely defined. It has been shown that an increase in phosphorylation of non-histone chromosomal proteins is associated with activation of chromatin for RNA synthesis (Stein et al., 1974; Johnson et al., 1974). Kostraba et al. (1975) have reported that phenol-soluble proteins of the

Table I: Rat Liver Nuclear Proteins Soluble at Low Ionic Strength.

Protein Spot No.[a]	0.15 M NaCl Extract	0.35 M NaCl Extract
B region 5L	+	+
6*	++++	+
10*	+++	+
10p*	+	+
18	+++	++
18p*	+	–
22	+++	+++
24	++++	+++
24p*	++++	+
25	++++	+++
26	++++	+++
33	++	+
33*	+++	++
E	+	–
J	+	–
P	++++	++
C region 1	+++	++
2p*	+	+
3p*	+++	+++
4	+	–
5	+	+++
5*	+	–
5p*	+	+
6	+	+
13	+	–
14	+	–
18	+	++++
18*	+	++++
25	+	–
A	+	+
B	++	+
B*	–	+
BL	+	+
C	++	+
C*	+	+
Cp*	+	–
G	+	+
H	+	+
H′	+	–
I	+	–
M	+	+++
N′	++	+
N′*	–	+
Q	+	++
T*	+	–
U*	+	–
X*	+	–

[a] * indicates ^{32}P-labeled spot. The most intense staining or ^{32}P-labeled spots are indicated by 4+.

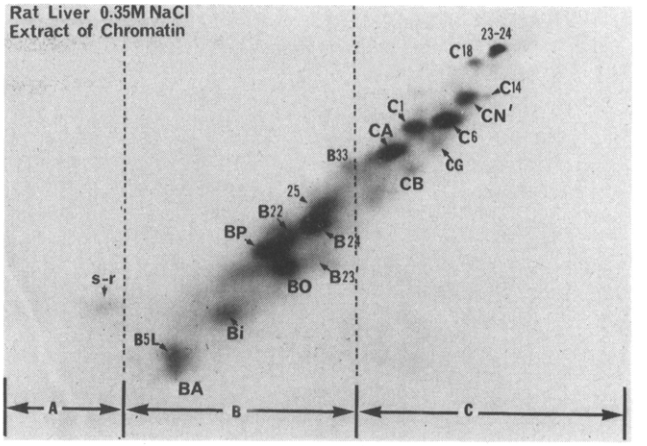

FIGURE 7: Two-dimensional gel electrophoresis of 0.35 M NaCl soluble proteins extracted from chromatin prepared from rat liver citric acid nuclei.

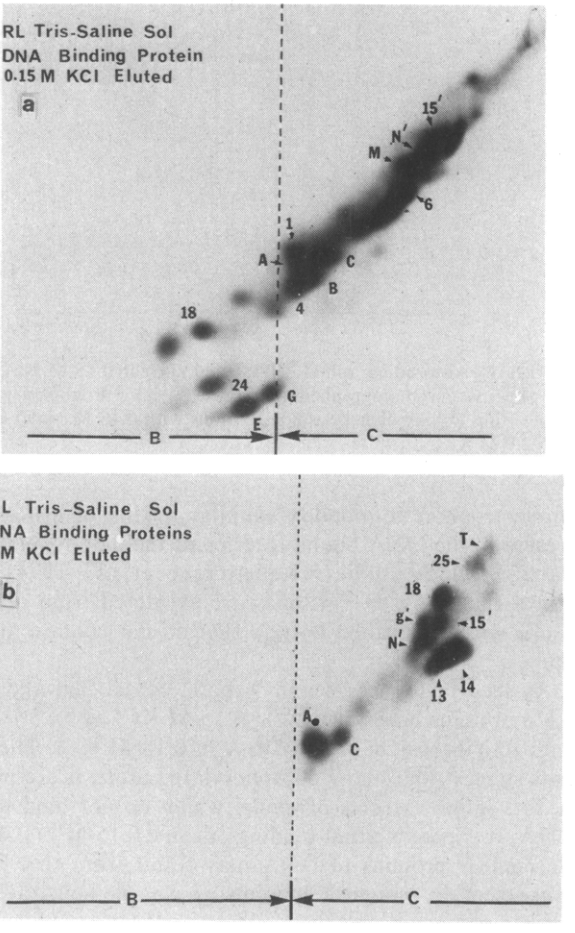

FIGURE 8: Two-dimensional gel electrophoresis of 0.15 *M* NaCl soluble nuclear proteins chromatographed on DNA–polyacrylamide columns as described in Materials and Methods. (a) 0.15 *M* NaCl soluble nuclear proteins that bound to DNA in 0.05 *M* Tris-HCl (pH 7.8) and 0.05 *M* KCl and were eluted with 0.15 M KCl. (b) Proteins that remained bound to DNA column in (a) and were subsequently eluted with 2 M KCl.

0.35 M NaCl extract of rat liver chromatin bind specifically to rat liver DNA and increase or decrease RNA transcription in vitro (Kostraba and Wang, 1975).

The results of the present study indicate that there are many similarities of phosphoprotein gel patterns from Tris-saline and 0.35 M NaCl extracts of nuclei. Many of the phosphoproteins extracted from nuclei with these solutions have the same electrophoretic mobilities as phosphoproteins tightly bound to chromatin and extracted with DNase treatment (Olson et al., 1975). These results suggest that phosphoproteins are bound to chromatin or DNA in the nucleus to varying degrees as are apparently the nonphosphorylated proteins.

Although many 0.15 M NaCl soluble nuclear proteins bind to DNA in vitro in 0.05 M KCl, few proteins bind to rat liver DNA in the presence of 0.15 M KCl (Figure 8b) which is a more physiological ionic strength. The major proteins of this DNA bound fraction have a molecular weight of 50 000–80 000 and are similar to DNA binding proteins of the 0.35 M NaCl soluble nuclear fraction. These proteins appear to have some specificity for rat liver DNA since they were not absorbed by *E. coli* DNA. Protein A18 which has

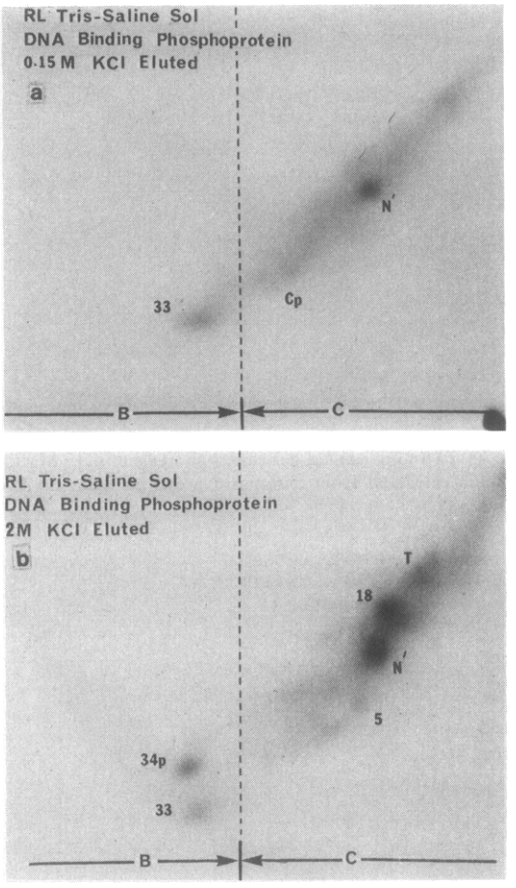

FIGURE 9: Autoradiograph of ^{32}P-labeled nuclear 0.15 M NaCl soluble proteins chromatographed in Figure 8. (a) ^{32}P-labeled proteins eluted from DNA polyacrylamide columns with 0.15 M NaCl and (b) with 2.0 M KCl.

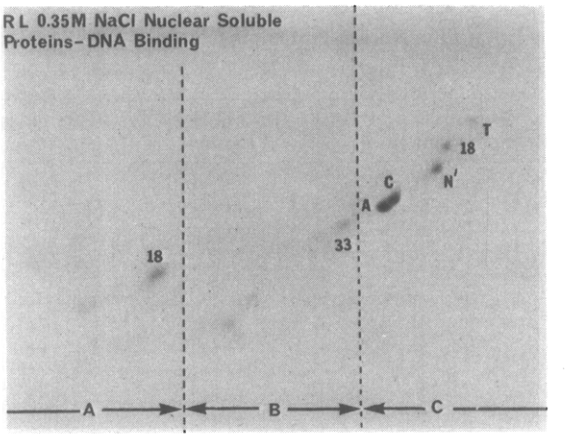

FIGURE 10: Two-dimensional gel electrophoresis stained pattern of 0.35 M NaCl soluble rat liver nuclear proteins that were bound to DNA in 0.15 M KCl and were eluted with 2 M KCl. Proteins were first passed through an *E. coli* DNA–polyacrylamide column in 0.15 M KCl–0.05 M Tris-HCl (pH 8.0). The unbound fraction was then chromatographed on a rat liver DNA–polyacrylamide column in 0.15 M KCl and the bound fraction was eluted with 2 M KCl.

Table II: Rat Liver Nuclear DNA Binding Proteins.

Protein Spot No.[a]	0.15 M NaCl Extract	0.35 M NaCl Extract
A region 18	–	+
B region 33*	+	+
34p*	+	–
C region 5*	+	–
13	+	–
14	+	–
15′	+	–
18*	+	+
25	+	–
A	+	●
C	+	+
g′	+	–
N′*	+	+
T*	+	+

[a] *indicates phosphoprotein. The DNA binding proteins compared are those of both the 0.15 M NaCl and 0.35 M NaCl extracts which bound to rat liver DNA in the presence of 0.15 M KCl and were then eluted with 2.0 M KCl.

an electrophoretic mobility smmilar to that of histone 1 is present in the DNA bound fraction of the 0.35 M NaCl extract. Previous studies (Kellermeyer et al., 1974) have shown that some H1 histones are extracted from rat liver nuclei with Tris-saline buffers that do not contain magnesium.

As seen in Figures 8 and 9, both ^{32}P-labeled and unlabeled proteins bind to DNA in 0.15 M KCl. Only 5% of the radioactivity was bound to DNA in 0.15 M KCl. These results suggest that many phosphorylated proteins are present in Tris-saline extracts of nuclei which do not bind to free DNA. It is possible that binding of some 0.15 M NaCl soluble nuclear proteins to DNA may result from specific deoxynucleotide sequence binding as was demonstrated for the lac repressor protein (Gilbert and Muller-Hill, 1967; Riggs et al., 1970) and as has been suggested for eukaryotes (Sevall et al., 1975). Such binding of non-histone nuclear proteins to regions of DNA may well be influenced by histones. Both conformational and charge effects of histone binding may be necessary for binding of other proteins to DNA in chromatin. Some alteration of protein–protein interactions in chromatin may occur by phosphorylation of non-histone nuclear proteins.

Most of the phosphoproteins extracted from rat liver nuclei withTris-saline buffer (pH 8.0) are present in low molecular weight slowly sedimenting complexes and only two or three phosphoproteins are associated with more rapidly sedimenting informofer particles. Recently, it has been shown that nuclear informofer particles contain endogenous protein kinase activity (Schweiger and Schmidt, 1974; Blanchard et al., 1975). Although the precise number of proteins present in nuclear informofer particles has not been clearly defined, it is generally agreed that two to four major proteins are present in 30S–40S nuclear particles (Samarina et al., 1968; Matringe and Jacob, 1972; McParland et al., 1972; Williamson, 1973). These correspond to proteins BP, B22, 24, 25, and 26 in Figure 5b. Several species of higher molecular weight proteins have been reported to be present in larger nuclear particles (Niessing and Sekeris, 1970, 1971; Pederson, 1974) and may correspond to the faint protein spots of 50 000–100 000 molecular weight of the C region (Figure 5b).

Since the phosphorylation of informofer proteins isolated from 30S nuclear particles and nuclear salt extracts seems to differ, the extent of phosphorylation of these proteins may be related to their functional state at a given time, i.e., whether they are actively transporting messenger precursor RNA out of the nucleus or whether they are involved in storage of this RNA.

The slowly sedimenting phosphoproteins of the 0.15 M NaCl soluble nuclear protein fraction may contain "cytonucleoproteins" similar to hormone receptor proteins which may be transported readily between nucleus and cytoplasm and involved in gene regulation. The effects of these proteins on RNA transcription in in vitro systems await the isolation and purification of individual species of these proteins.

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