Purification of a Phosphoprotein from Chromatin of Rat Liver

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A simple and effective method to purify a phosphoprotein (B₂) (Mr, 68 000, pI 6.2–8) from phenol-soluble non-histone chromatin proteins of rat liver is described. The purification involved only two steps, CM-cellulose chromatography and preparative SDS/polyacrylamide (10%)-gel electrophoresis. The purified phosphoprotein B₂ was shown to be homogeneous by SDS/polyacrylamide-gel electrophoresis. The yield was 2% of total non-histone chromatin proteins. The acidic to basic amino acid ratio of phosphoprotein B₂ was less than 1, with high contents of glutamic acid, aspartic acid, arginine, lysine, glycine and alanine. The phosphate content of this protein is 0.3%.

Studies on chromatin reconstitution in vitro have indicated that non-histone chromatin proteins are associated with tissue-specific template activity as well as the specific control of gene expression (for reviews see: Stein et al., 1974; MacGillivray & Rickwood, 1975; Chiu & Hnilica, 1977; Bekhor, 1978; Liew, 1979). Evidence also suggests that phosphorylation of non-histone chromatin proteins is involved in the regulation of gene expression (Stein et al., 1974; Kleinsmith et al., 1976; Jungmann & Russell, 1977). For example, phosphorylation and dephosphorylation of non-histone chromatin proteins were found to be associated with cellular activity (Kleinsmith et al., 1966; Gornall & Liew, 1974; Ezrailson et al., 1976; Thomson et al., 1977) and the phosphorylation of nuclear proteins during hormone action (Jungmann & Schweppe, 1972; Liew et al., 1973). It was also found that phosphoproteins bound selectively to homologous DNA (Teng et al., 1971; Allfrey et al., 1973) and enhanced the RNA synthesis in cell-free systems (Teng et al., 1971; Kostraba et al., 1975). Nevertheless, most of the work was done on a group of non-histone chromatin proteins which was enriched in phosphoprotein. In order to understand the precise role(s) of phosphoprotein in relation to gene regulation, specific phosphoprotein(s) should be isolated and characterized.

Kleinsmith (1973) isolated a group of nuclear phosphoproteins by DNA-cellulose chromatography. Kostraba et al. (1975) also succeeded in isolating a group of enriched non-histone nuclear phosphoproteins from Ehrlich ascites-tumour cells by salt and phenol extraction. MacGillivray et al. (1978) isolated a low-molecular-weight phosphorylated component of non-histone chromatin proteins by gel chromatography and electrophoresis from mouse nuclei. Other low-molecular-weight non-histone chromatin proteins (Mr ≥ 30 000) were also isolated by several laboratories (Patel & Holoubek, 1974; Goldknopf et al., 1975; Herrick & Alberts, 1976; Bluthmann, 1976). However, isolation of a specific non-histone nuclear phosphoprotein with relatively high molecular weight has yet to be achieved. In the present paper we describe a very simple method to isolate the specific phosphoprotein B₂, which has previously been shown to be associated with the chromatin subunits of rat liver (Liew & Chan, 1976; Chan & Liew, 1977).

Materials

Chemicals

Acrylamide, NN'-methylenbisacrylamide and NNN'N'-tetramethylethylenediamine were purchased from Eastman Organic Chemicals, Rochester, NY, U.S.A.; ultrapure urea and Tris base were obtained from Schwarz/Mann Co., Orangeburg, NY, U.S.A., and CM23 CM-cellulose was obtained from W. and R. Balston, Maidstone, Kent, U.K. All other chemicals were of reagent grade.

Animals

Male albino rats weighing about 200 g were used. They had access to tap water and were fed on Purina Laboratory Chow. Food was removed the night before experiments.

Radioisotopes

[32P]Phosphoric acid (carrier-free) was purchased from New England Nuclear Corp., Boston, MA, U.S.A.
Methods

Preparation of phenol-soluble non-histone chromatin proteins

Rat liver nuclei were prepared as described previously (Chan & Liew, 1977). The phenol-soluble non-histone chromatin proteins were isolated by the method described by Teng et al. (1971). Briefly, the purified liver nuclei were suspended and stirred for 15 min in 0.14 M-NaCl (2 ml/g of liver tissue), followed by centrifugation at 3000 g for 5 min. This extraction was then repeated. Histones were then removed by extracting twice with 0.25 M-HCl (2 ml/g of liver tissue). Lipids were removed by a chloroform/methanol mixture. The non-histone chromatin proteins were then suspended in TEM buffer (0.1 M-Tris/HCl, pH 8.4, 0.01 M-EDTA, 0.14 M-β-mercaptoethanol), and an equal volume of phenol saturated with TEM buffer was added. The mixture was stirred overnight in the cold-room, followed by centrifugation at 12000 g for 10 min. The aqueous phase was collected and re-extracted with the buffered phenol as described previously. The two phenol extracts were combined and dialysed against 0.1 M-acetic acid containing 0.14 M-β-mercaptoethanol until the phenol phase was decreased to one-fifth of its original volume. The phenol extract was subsequently dialysed against 0.05 M-acetic acid/9.0 M-urea/0.14 M-β-mercaptoethanol for 24 h. Dialysis was continued in 0.1 M-Tris/HCl (pH 8.4)/8.6 M-urea/0.01 M-EDTA/0.14 M-β-mercaptoethanol for 2 h, followed by an equilibrating buffer consisting of 8 M-urea, 0.02 M-glycine, 0.02 M-Tris/HCl, pH 9.2, 0.05 % β-mercaptoethanol and 0.1 mm-phenylmethanesulphonyl fluoride.

One-dimensional SDS/polyacrylamide-gel electrophoresis

Chromatin proteins (1–2 mg/ml) were solubilized by adding an equal volume of a mixture containing 5% SDS, 1% β-mercaptoethanol and 65 mM-Tris/HCl, pH 6.8. The SDS/polyacrylamide (10%) gel electrophoresis was carried out as described by Laemmli (1970). The separating gels (0.6 cm × 6.5 cm) contained 10% acrylamide, 0.27% N,N'-methylenebisacrylamide, 0.375 M-Tris/HCl, pH 8.8, 0.1% SDS, 0.03% ammonium persulphate and 0.05% NNN'N'-tetramethylethylenediamine. The separating gel was overlaid with 0.1 ml of water and left overnight at room temperature before polymerization of the stacking gel. The stacking gels (0.6 cm × 0.5 cm) contained 3% acrylamide, 0.08% N,N'-methylenebisacrylamide, 0.125 M-Tris/HCl, pH 6.8, 0.1% SDS, 0.03% ammonium persulphate and 0.05% NNN'N'-tetramethylethylenediamine. The stacking gel was overlaid with water and allowed to polymerize for 1 h before electrophoresis. About 50 μg of protein was layered on the stacking gel. Gel electrophoresis was performed at a constant current of 1 mA/tube until the tracing dye (Bromophenol Blue) had reached the bottom of the gel. The gels were fixed with a fixing solution consisting of 50% (v/v) methanol and 10% (v/v) acetic acid and stained with 0.1% Coomassie Brilliant Blue in the fixing solution. The gels were destained with a solution of 5% methanol and 10% acetic acid.

Isolation of the phosphoprotein B2 by SDS/polyacrylamide-gel electrophoresis

The enriched phosphoprotein fraction obtained from CM-cellulose column chromatography was dialysed overnight against two changes of equilibrating buffer, pH 9.2, to remove salt. The protein sample was then concentrated to a final protein concentration of about 1 mg/ml by Sephadex G-200 (concentration of the protein by ultrafiltration resulted in a low yield). To this fraction an equal volume of a mixture containing 5% SDS, 1% β-mercaptoethanol and 65 mM-Tris/HCl, pH 6.8, was added. The protein was fractionated by SDS/polyacrylamide (10%)-gel electrophoresis. After electrophoresis, the gels were immersed in 1 M-KCl for 3 min (Nelles & Bamburg, 1976). The protein bands in the gel were then made visible by precipitation of protein–SDS complexes after overnight storage in the refrigerator. Gel segments corresponding to the phosphoprotein B2 were excised and ground into small pieces by a glass rod. The gel pieces were soaked in a mixture containing 5% SDS, 1% β-mercaptoethanol and 65 mM-Tris/HCl, pH 6.8. The gel mixture was then poured into a glass tube which contained a short polymerized SDS/polyacrylamide (10%) gel (supporting gel, 1 cm × 1.5 cm) at the end of the tube. A dialysis-
tubing sac was tied at the anodic end under the supported gel. Electrophoresis was performed at 5 mA/tube for 48 h. The protein was collected in a dialysis-tubing sac.

**Two-dimensional polyacrylamide-gel electrophoresis**

Chromatin proteins were subjected to isoelectric-focusing in the first dimension and subsequently fractionated by SDS/polyacrylamide-gel electrophoresis in the second dimension by the method described previously (Suria & Liew, 1974; Jackowski et al., 1976; Chan & Liew, 1977). The Coomassie Blue-stained two-dimensional slab gels were dried under vacuum overnight. The 32P-labelled non-histone chromatin proteins were detected by radioautography by using Kodak X-ray film (NS-54T). The gels were exposed to X-ray film for 2–3 days before being developed.

**Protein determination and analysis**

Protein determination followed the method described by Lowry et al. (1951). Bovine serum albumin was used as the standard.

The amino acid composition of the phosphoprotein was determined by an ion-exchange amino acid analyser. The protein was hydrolysed in 6M-HCl at 105°C for 21 h before analysis.

**P, determination**

The phosphate content of phosphoprotein B2 was determined by the method of Martin & Doty (1949). The phosphoprotein B2 eluted from the SDS/polyacrylamide gel was precipitated with an equal volume of 25% (w/v) trichloroacetic acid and then with ether at 0°C. The phosphoprotein was hydrolysed with 1.0M-NaOH at 100°C for 15 min. A sample (10 μl) was taken for protein determination. The remainder was treated with ammonium molybdate and then extracted with 2-methylpropan-1-ol/ benzene (1:1, v/v). The phosphomolybdate complex was measured at 660 nm in the presence of SnCl2.

**Results**

An attempt was made to isolate a specific phosphoprotein B2, which has previously been identified in chromatin subunits (Liew & Chan, 1976; Chan & Liew, 1977). However, to minimize the time required for the purification procedure, the preparation of chromatin subunits was omitted and phenol-soluble non-histone chromatin proteins were isolated directly from the nuclei. The non-histone chromatin proteins of rat liver were then fractionated by CM-cellulose column chromatography. The elution profile is shown in Fig. 1. Two fractions were obtained. In five separate experiments, about 60% (59.2 ± 3.8%) of

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the proteins were not bound to the column. The 'bound' fraction (27.5 ± 6.0%) was then eluted from the column by 0.3 M-NaCl in the equilibrating buffer. This fraction was then rechromatographed under similar conditions, but with an increase in pH from 9.2 to 10.0. About 14% of the starting non-histone chromatin protein was eluted as the 'unbound' fraction and about 7% was finally eluted as the 'bound' fraction, which contained the phosphoproteins. About 86% of the non-histone chromatin proteins were accounted for after column chromatography.

Fig. 2 shows the one-dimensional SDS/polyacrylamide-gel pattern of fractions obtained from CM-cellulose chromatography. As compared with the phenol-soluble non-histone chromatin proteins (Fig. 2, gel a), it was found that the unbound fraction (Fig. 2, gel b) contained most of the total non-histone chromatin proteins. However, the specific phosphoprotein B2 (arrow) and some other proteins (Fig. 2, gel c) were bound to the column. Further purification of the phosphoprotein B2 was achieved by rechromatography of the bound fraction in the CM-cellulose column at a higher pH (pH 10). Under this condition most of the proteins in the unbound fraction were removed, leaving the phosphoprotein B2 and two other major proteins (Fig. 2, gel d) bound to the CM-cellulose. These proteins (enriched phosphoprotein fractions) were again eluted by 0.3 M-NaCl in the equilibrating buffer.

The complexity of the non-histone chromatin proteins was revealed by the high resolving power of the two-dimensional polyacrylamide-gel electrophoresis. As shown in Fig. 3(a), the phenol-soluble non-histone chromatin proteins could be fractionated into more than 100 components with molecular weights ranging from 30000 to larger than 100000 and pI from 5 to 9.5. The isolation of specific phosphoprotein B2, which is shown in the rectangle, was carried out. Most of the non-histone chromatin proteins (Fig. 3b) did not bind to the CM-cellulose column under the experimental conditions, except

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**Fig. 3. Fractionation of phenol-soluble non-histone chromatin proteins and purified phosphoprotein by two-dimensional polyacrylamide-gel electrophoresis**

For full details, see the Methods section. (a) Phenol-soluble non-histone chromatin proteins. (b) 'Unbound' fraction from the CM-cellulose chromatography, which contains the majority of non-histone chromatin proteins except the phosphoprotein B2 and other basic proteins. (c) 'Bound' fraction from the CM-cellulose chromatography, which contains the phosphoprotein B2 (rectangle) and other basic proteins of non-histone chromatin proteins. (d) Enriched phosphoprotein fraction, which was obtained from the rechromatography of the 'bound' fraction on the CM-cellulose column at pH 10. The phosphoprotein B2 and two other basic proteins were observed.
for phosphoprotein B₂ and a few other proteins. Furthermore the non-histone chromatin proteins retained in the CM-cellulose column were relatively basic, with isoelectric points greater than 7 (Fig. 3c). Further purification of the phosphoprotein B₂ by rechromatography removed some of these basic proteins. The two-dimensional electrophoresis pattern of the enriched phosphoprotein fractions is shown in Fig. 3(d). Only three groups of non-histone chromatin proteins were observed. It should be noted that the phosphoprotein B₂ was resolved into five or six distinct fractions by this two-dimensional gel system.

Fig. 4 shows the radioautograph of the ³²P-labelled non-histone chromatin proteins on the two-dimensional gel. Fig. 4(a) shows that most of the phosphoproteins were not bound to the CM-cellulose column. In general there were three major groups of phosphorylated non-histone chromatin proteins, with mol.wts. around 20000, 38000 and 68000 in the phenol-soluble proteins (Fig. 4b). The phosphoprotein B₂ belongs to the high-molecular-weight group (Mr 68000). However the phosphorylated protein B₂ and two other slightly phosphorylated proteins were retained in the CM-cellulose column (Fig. 4c) and could be removed by 0.3M-NaCl in the equilibrating buffer. As shown in Fig. 4(d), all these phosphoproteins were again retained in the CM-cellulose column during rechromatography at the higher pH.

Before the use of CM-cellulose chromatography, a DNA affinity column had been used to fractionate the phenol-soluble non-histone chromatin proteins. Rat liver DNA was incorporated into the CM-cellulose (2.5 mg of DNA/ml of DNA-CM-cellulose) and the affinity chromatography was performed by the method described by Potuzak & Wintersberger (1976). However, the protein recovery after passage through the column was very low (10–30%). In addition, the two-dimensional polyacrylamide-gel
electrophoresis pattern of the fractionated protein after column chromatography was not reproducible. It was later found that the addition of urea to the equilibrating buffer could increase the protein recovery from the column and provide a more reproducible result. Aggregation of the non-histone chromatin proteins either to the matrix of the cellulose or to themselves in the absence of urea was suspected to be the reason for the low recovery. Nevertheless, it was found that chromatography using CM-cellulose and DNA–CM-cellulose gave similar results under the high-urea condition. Therefore, the simple and effective CM-cellulose chromatography was used.

During the development of the chromatographic conditions for the CM-cellulose column, it was found that less protein was retained in the column at a higher pH (cation exchanger). However, the specific phosphoprotein $B_2$ with pI 6.2–8 was still bound to the column. This property facilitated its purification by the CM-cellulose column. An attempt was also made to elute the phosphoprotein ($B_2$) from the column by using a salt gradient (0–0.3 M-NaCl). Two fractions overlapping each other were obtained. However, both fractions contained phosphoprotein $B_2$. In an alternative method, the proteins retained in the column were eluted by the pH10 buffer followed by urea buffer containing 0.3 M-NaCl. Very little protein was eluted by the pH10 buffer. Therefore rechromatography at a higher pH was required for better purification of the phosphoprotein $B_2$.

Based on the scanned gel area (Fig. 5a), it was
found that the percentage of phosphoprotein B2 in the enriched phosphoprotein fraction was about 23%. Taking into account the protein recovery of the previous steps, it was estimated that the phosphoprotein B2 constituted about 2% of the phenol-soluble non-histone chromatin proteins.

The phosphoprotein B2 was finally isolated by excising the protein band from the SDS/polyacrylamide (10%) gel and eluted electrophoretically as described in the Methods section. The protein recovery of this final step was about 70%. Table 1 shows the yield of the phosphoprotein B2 that could be obtained from 1 g of rat liver tissue. The isolated phosphoprotein B2 was subjected to SDS/polyacrylamide (10%)-gel electrophoresis. As shown in Fig. 5(b), only one band was observed, indicating that the isolated phosphoprotein B2 was pure.

The amino acid composition of the phosphoprotein B2 is shown in Table 2: the protein was enriched in glutamic acid, aspartic acid, arginine, lysine, glycine and alanine. The ratio of the sum of aspartic acid and glutamic acid to the sum of lysine, histidine and arginine was 0.93, indicating that the molecule was an unusual polypeptide of non-histone chromatin proteins. The N-terminal of the phosphoprotein B2 was determined by the method described by Weiner et al. (1972). Preliminary results revealed that the amino group of the N-terminal amino acid was blocked.

The phosphate content of non-histone chromatin proteins and phosphoprotein B2 was also determined, as shown in Table 3; the P1 content of phosphoprotein B2 was 0.3%. We previously showed (Chan & Liew, 1976) that the isolated phosphoprotein B2 was pure.

Table 1. Protein recovery of the phosphoprotein B2 from rat liver
Liver tissue (1 g)

Nuclear proteins (5.5–6 mg)

Phenol-soluble non-histone chromatin proteins (0.19–0.25 mg)

CM-cellulose chromatography

Enriched phosphoprotein fraction (12.6–16.4 μg)

SDS/polyacrylamide (10%)-gel electrophoresis

Phosphoprotein B2 (2–4 μg)

Table 2. Total amino acid composition (mol %) of phosphoprotein B2
Values for proteins HMG1 and HMG2 are taken from Walker et al. (1976).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Phosphoprotein B2</th>
<th>HMG 1</th>
<th>HMG 2</th>
</tr>
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<td>Asp</td>
<td>8.9</td>
<td>11.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Thr</td>
<td>3.3</td>
<td>2.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Ser</td>
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<td>Pro</td>
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<tr>
<td>Ala</td>
<td>9.3</td>
<td>9.1</td>
<td>7.5</td>
</tr>
<tr>
<td>Val</td>
<td>1.9</td>
<td>2.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Cys</td>
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<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Met</td>
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</tr>
<tr>
<td>Arg</td>
<td>13.2</td>
<td>3.9</td>
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N-Terminal amino acid

Blocked (?) Gly Gly/Lys

Table 3. Determination of P1 content of phosphoprotein B2

<table>
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<th>Protein (μg)</th>
<th>Phosphate (nmol)</th>
<th>(%) w/w</th>
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<tr>
<td>Non-histone chromatin proteins</td>
<td>760</td>
<td>37.5</td>
</tr>
<tr>
<td>Phosphoprotein B2</td>
<td>320</td>
<td>10.0</td>
</tr>
</tbody>
</table>
1977) that most of the phosphate in phosphoprotein B₂ was covalently linked to serine residues, and a minor amount of phosphothreonine was also detectable.

Discussion

A simple method to isolate the specific phosphoprotein B₂ from the phenol-soluble non-histone chromatin proteins has been described. The procedure is easy to operate and the result is highly reproducible. It involves only two steps, CM-cellulose chromatography and SDS/polyacrylamide (10\%) gel electrophoresis. CM-cellulose chromatography at high pH provided an effective way to purify this protein. As revealed by two-dimensional gel electrophoresis, CM-cellulose chromatography removed over 90\% of the non-histone chromatin proteins, which were devoid of the specific phosphoprotein B₂. Radioautography of the bound and unbound ³²P-labelled non-histone chromatin proteins on two-dimensional gels also confirmed that there was no cross-contamination (Fig. 4). About 2–4 μg of the purified phosphoprotein B₂ could be obtained from 1 g of rat liver tissue (Table 1). The purified phosphoprotein B₂ was shown to be homogeneous by one-dimensional SDS/polyacrylamide-gel electrophoresis (Fig. 5b). However, five distinct subfractions were revealed by two-dimensional polyacrylamide-gel electrophoresis, which may reflect varying degrees of phosphorylation.

The heterogeneity and extent of phosphorylation of each phosphoprotein B₂ subfraction remains to be investigated. The heterogeneity of the purified chromatin fractions of the high-mobility-group (HMG) proteins was also observed by Johns and his co-workers (Goodwin et al., 1976, 1977; Walker et al., 1976). They found that protein HMG2 could be resolved into four fractions by isoelectrofocusing, whereas protein HMG1 exhibited a complex pattern. The multiple spots of protein that focused in the alkaline region of the isoelectrofocusing gel are probably not due to modifications in vitro arising from interactions between the Ampholines and the protein molecules (e.g. Gineitis et al., 1978). As shown in Fig. 4, only certain proteins (e.g. B₂) exhibited multiple spots, whereas others did not; it follows that this isoelectric heterogeneity was not a general artefact. Furthermore, we reported (Suria & Liew, 1979) that heterogeneous ribonucleoprotein particles solubilized directly in urea sample buffer without being subjected to the phenol-extraction procedure revealed an identical pattern of multiple spots. An alternative explanation might be that modification of proteins is due to phosphorylation (or ADP-ribosylation in vivo). It has been shown that phosphorylation of proteins modifies the charge of the protein molecules and that the various charged species are resolved as multiple spots on the isoelectrofocusing gel. A phosphoprotein identified by MacGillivray et al. (1978), using salt extraction, hydroxyapatite chromatography and two-dimensional polyacrylamide-gel electrophoresis, was observed to be very similar to our findings in terms of molecular weight and isoelectric points. This finding lends further support to the fact that this phosphoprotein has isoelectric heterogeneity.

The specific phosphoprotein B₂ was shown to be associated with the chromatin subunits of the rat liver (Chan & Liew, 1977). A similar result was also observed by Bohm et al. (1977), who reported on the presence of a phosphoprotein with \( M_c \), 70000 in the chromatin subunits of ascites cells. There are only a few non-histone chromatin proteins that have been identified and characterized in chromatin subunits, e.g. HMG proteins by Goodwin et al. (1977), A-24 protein by Busch and his associates (Goldknopf et al., 1977), a specific protein kinase by Simpson (1978) and DNA polymerase β by Schlaeger et al. (1978). It is also noteworthy that phosphoprotein B₂ is not likely to be one of the subunits of RNA polymerase (Seifart et al., 1972; Kedinger et al., 1974; Goldberg et al., 1977), since none of the subunit species of RNA polymerase from rat liver have the same molecular-weight range as this phosphoprotein [e.g. molecular weights of rat liver RNA polymerase I subunits (Goldberg et al., 1977) are 205000, 125000, 51000, 44000, 26000 and 16000]. Busch and his co-workers isolated C-14 protein with \( M_c \), 70000 from Novikoff-hepatoma ascites. This protein, however, did not incorporate ³²P₁ (Gordon et al., 1977). It was also found that phosphoprotein B₂ is not one of the 40S-ribosomal-subunit proteins (Karn et al., 1977; Beyer et al., 1977). The amount of phosphoprotein B₂ in the chromatin subunits as compared with histone is very low. This suggests that phosphoprotein B₂ may not be an essential element of nucleosome structure, but may be one of the regulatory proteins in the chromatin of rat liver. In particular, the unusual amino acid composition of the B₂ protein, which has both high glutamic and aspartic acid contents and a high proportion of basic amino acids, arginine and lysine, suggests a polarization of charge which would confer both DNA-binding and histone-binding properties, as is the case for the HMG proteins (Goodwin et al., 1976, 1977; Walker et al., 1976).

Nuclei from 1 g of rat liver tissue contained 1.5 mg of DNA, which corresponded to about 11.5 nmol of chromatin subunits (molecular weight of 200 basepairs of DNA is 130000). About 0.2 mg of phenol-soluble non-histone chromatin proteins was obtained/g of liver tissue, from which about 4 μg (2% of 0.2 mg) or 0.059 nmol of phosphoprotein B₂ (\( M_c \), 68000) could be isolated. From this calculation, it was estimated that at least one molecule of phosphoprotein B₂ occurs per average of 200 chromatin subunits. Because phosphoprotein B₂ has been identified
in purified nucleosome monomers released by micrococcal-nuclease digestion (Chan & Liew, 1977; Liew & Chan, 1976), this finding also suggests that the phosphoprotein B2 is associated with a subset of chromatin subunits. The size of the eukaryotic genome is about 3 x 10¹⁵ daltons, in which only 5–10% is expressed (Rabbits, 1975; Matthews, 1975). If the phosphoprotein is involved in gene expression, it is expected that it should be localized in the active region of the genome. The proportion of phosphoprotein B2 in chromatin (1 per every 200 chromatin subunits) is not inconsistent with a localization in transcriptionally active regions.

In conclusion, much evidence suggested that phosphoproteins are involved in regulation of gene expression (Kleinsmith et al., 1966; Teng et al., 1971; Jungmann & Schweppe, 1972; Johnson & Allfrey, 1972; Liew et al., 1973; Shea & Kleinsmith, 1973; Johnson et al., 1974; Steen et al., 1974; Kostraba et al., 1975; Ezrailson et al., 1976; Thomson et al., 1977). In particular, it was found that RNA polymerases are subject to phosphorylation (Jungmann et al., 1974). Most of the earlier work was done on a complex group of non-histone chromatin proteins, enriched in phosphoprotein content. To gain a better understanding of the mechanism and the role of phosphoprotein in gene expression of the eukaryotic cell, isolation of a specific phosphoprotein is essential. We hope that future experiments on this phosphoprotein B2 will provide a better insight into its localization and role in the mechanism of gene expression.

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