PHOSPHORYLATION OF NON-HISTONE CHROMATIN PROTEINS IN NORMAL AND REGENERATING RAT LIVER, NOVIKOFF HEPATOMA AND RAT HEART

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1. Introduction

Phosphorylation of non-histone chromatin proteins (NHP) has been related to gene control in numerous cell systems [1–7]. Phosphorylation of NHP changes with phases of the cell cycle and appears to be correlated with synthesis of RNA and DNA [8,9]. Since most of the RNA synthesized in growing tissues is ribosomal [10], possible correlations exist between growth rate and ^{32}P labeling of nucleolus-specific NHP [11]. The present study was designed to compare ^{32}P labeling of chromatin NHP of growing and nongrowing cells. At least two proteins, C18 and Cg′, incorporate relatively greater amounts of ^{32}P in regenerating liver and Novikoff hepatoma which have growing and dividing cells than in normal liver and heart. The major ^{32}P-labeled NHP were common to the four tissues.

2. Materials and methods

2.1. *In vivo incorporation of $^{32}PO_4$ – preparation of tissues*

Carrier-free $^{32}P_i$ neutralized with 1 N NaOH and diluted to approx. 70 mCi/ml was injected intraperitoneally into 200 g male albino rats obtained from the Holtzman Rat Company (Madison, Wisc.) 2 h prior to killing the rats. Livers were perfused with a solution (0–4°C) of 0.13 M NaCl, 0.005 M KCl, 0.008 M $MgCl_2$ and 1 mM PMSF (NKM). For studies on regenerating liver, partial hepatectomy (70% of liver mass) [12,13] was performed 18 h prior to injection of $^{32}P_i$. Rats bearing Novikoff hepatoma cells were used six days after transplantation. The procedure for ^{32}P labeling of Novikoff hepatoma cells in vitro was previously described [11,14].

2.2. *Preparation of nuclei and chromatin*

Solid tissues were minced at 0–4°C and passed through a Harvard tissue press. Nuclei were prepared by the method of Taylor et al. [15] with addition of 0.04 M potassium fluoride to the sucrose solutions. To prepare chromatin, purified nuclei were extracted as previously described [11].

2.3. *DNase digestion and preparation of samples for electrophoresis*

Chromatin extracted twice with 0.4 N H_2SO_4 [11] was suspended by homogenization in 2 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 M Tris, pH 7.5. DNase I digestion and subsequent steps in the preparation of proteins were done according to the method of Yeoman et al. [16] as modified by Olson et al. [11]. Two-dimensional polyacrylamide gel electrophoresis was performed as in previous studies [11,17].

2.4. *Detection of ^{32}P-labeled proteins*

The gel slabs were dried for 24 h on a Hoeffer Scientific SE-540 Gel Dryer (San Francisco, Calif.) and then taped to sheets of RP Royal X-omat film. The film was exposed from 1–14 days.

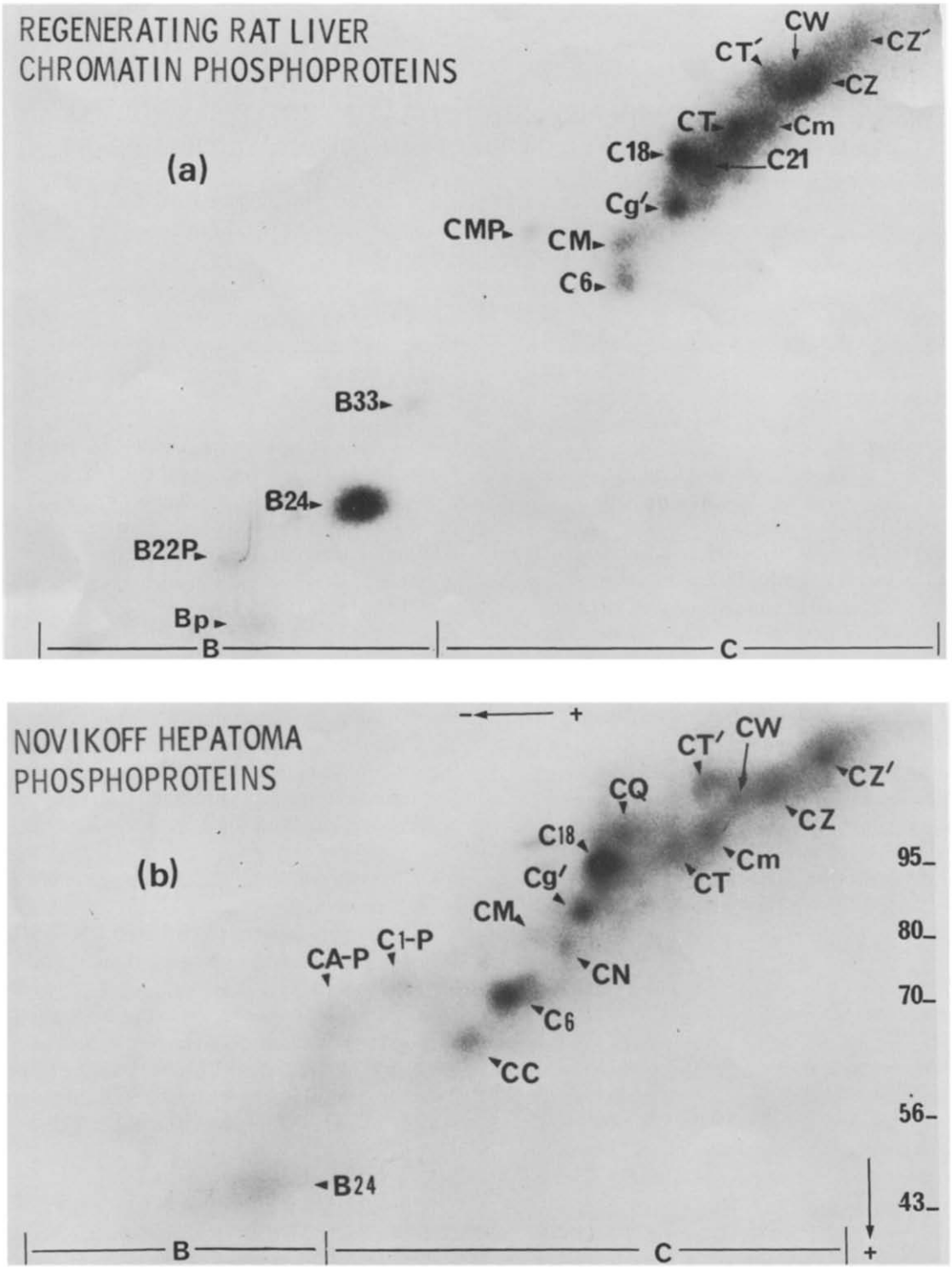
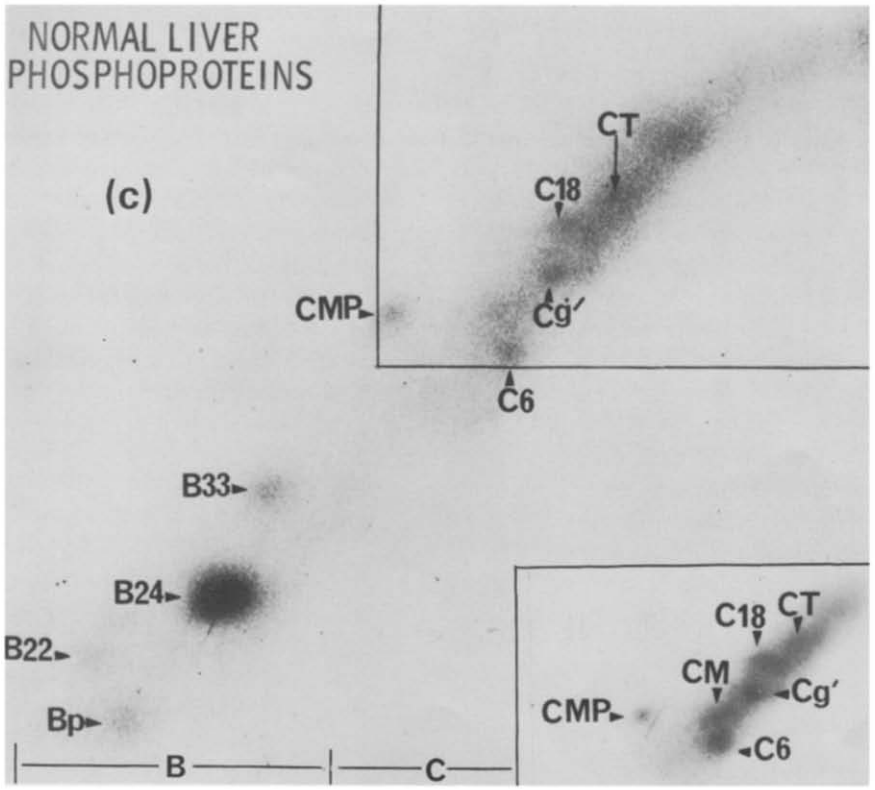


Fig.1. For legend see p. 72.



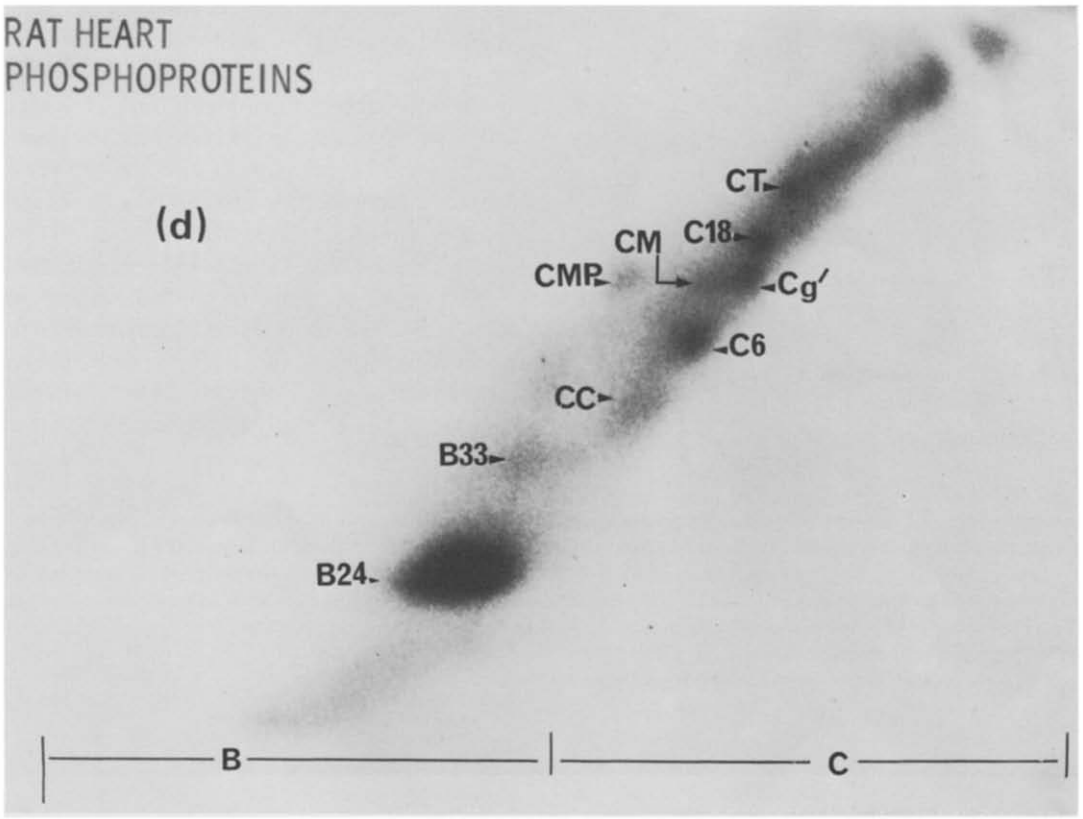


Fig.1. For legend see p. 72.

3. Results

Analysis of non-histone Chromatin Fraction II proteins of 18 h regenerating liver by two-dimensional electrophoresis and autoradiography showed that labeled phosphate was incorporated into 10 distinct protein spots (fig.1a). The most highly labeled was spot B24. Other B region spots, B22, Bp and B33, had lesser incorporation of ^{32}P (table 1). There was a general absence of label in the upper B region of this pattern as well as in the patterns from other tissues (fig.1b–d). C-region proteins C18, C21, Cg′ and CZ had a relatively high ^{32}P uptake. Lesser amounts of label were found in spots C6, CM, CT′ and CW.

Spots C6, Cg′, C18 and CZ′ were the most highly ^{32}P-labeled proteins in the two-dimensional pattern of Novikoff hepatoma Chromatin Fraction II (fig.1b). Several upper C region components, CM, CT, CT′, CW and CZ, were labeled to a lesser degree. Spot CQ was labeled in this pattern but not in regenerating liver. Spot B24 is only lightly labeled in the Novikoff hepatoma pattern.

In normal liver (fig.1c), the B region labeling pattern was similar to that of regenerating liver. Spot B24 was highly labeled and other spots, Bp, B22 and B33, incorporated lesser amounts of ^{32}P. Spots CMP, C6, Cg′, C18 and CT incorporated lesser amounts of ^{32}P. These spots were seen more clearly when the autoradiograph was exposed for four days (inset) rather than one day.

The ^{32}P autoradiograph of rat heart (fig.1d) was generally similar to the normal liver pattern; spot B24 was very dense. Spots C6, Cg′ and C18 were distinct spots with lesser amounts of label.

Table 1
Relative ^{32}P uptake into major phosphoproteins

	Reg. Liver	Novikoff Hepatoma	Normal Liver	Heart
CZ′	+	++	–	–
CZ	++	++	tr	tr
CT′	+	++	tr	tr
CT	++	++	+	+
CQ	tr	++	–	–
C18	+++	+++	+	++
C21	++	+	tr	tr
Cg′	+++	+++	+	++
C6	++	+++	+	++
B24	++++	+	++++	++++

Relative labeling of spots on autoradiographs were classified according to the following convention: Very large dense spots, ++++; smaller but dense spots, +++; less dense spots, ++; much less dense but distinct spots, +; faint or diffuse spots were designated by 'tr'.

4. Discussion

Strikingly, in Chromatin Fraction II a number of phosphorylated non-histone proteins (C6, Cg′ and C18) are common to the four tissues studied. Of these, C18 and Cg′ were particularly dense in the growing cells of the Novikoff hepatoma and regenerating liver. Protein C18 was previously shown to be increased in growing tissues [16]. Since spots C18 and Cg′ were also shown to be nucleolus specific [11], the increased uptake of label into these spots in regenerating liver [18] and Novikoff hepatoma may be correlated with the increased rate of rRNA synthesis. Although the function of these proteins is unknown, they may be associated with the synthetic machinery for production of preribosomal particles. These ^{32}P-incorporating proteins may be related to those previously observed in regenerating liver [2] and in concanavalin A stimulated lymphocytes [19].

An interesting phenomenon observed was that some radioactive spots varied slightly in mobility from tissue to tissue. For example, spots B24 and C6 moved more slowly in the first dimension in normal liver and heart. This may be the result of differences

Fig.1. Autoradiographs of two-dimensional polyacrylamide gel electrophoretogram of Chromatin Fraction II phosphoproteins. Samples were run in the first dimension (horizontal arrow) on disc gels of 6% polyacrylamide 6 M urea, 0.9 N acetic acid at 120 V for 6 h. For the second dimension (vertical arrow), an 8% polyacrylamide, 0.1% SDS slab gel was run for 14 h at 50 mA/slab. Gels were stained with Coomassie brilliant blue R, dried and subjected to autoradiography on X-omat X-ray film. Numbers at right of fig.1b indicate molecular weights in thousands as determined by molecular weight standards (bovine albumin, ovalbumin and chymotrypsinogen). (a) Regenerating liver, 1 day exposure. (b) Novikoff hepatoma, 1 day exposure. (c) Normal liver, 1 day exposure. The inset shows C region after a four-day exposure. (d) Rat heart, 1 day exposure.

in numbers of phosphate residues per molecule which may alter electrophoretic mobilities.

Qualitative differences were observed only in minor spots. Spot CMP was found in all patterns except Novikoff hepatoma. Spot CQ and the lesser spots CA-P and C1-P were only found in Novikoff hepatoma.

Although tissue specificity of nonhistone phosphoproteins has been noted [7,20], this and another study [21] suggest limited qualitative tissue specificity. However, this system probably cannot detect tissue specific gene control proteins present in very low concentrations. More sensitive techniques for isolation and assay of transcriptional effects of phosphorylated and dephosphorylated molecules should aid in further elucidation of their roles in gene regulation.

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