RAT LIVER NON-HISTONE PROTEINS : CORRELATION BETWEEN PROTEIN KINASE ACTIVITY AND ACTIVATION OF RNA SYNTHESIS.

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SUMMARY

Rat liver non-histone proteins that stimulate chromatin-templated transcription have protein kinase activity. They catalyze phosphorylation of histone and of chromatin phosphoprotein. A correlation between protein kinase activity and stimulation of chromatin-templated RNA polymerase reaction has been observed.

One of the authors (M.K.) reported previously (1) that the non-histone chromosomal proteins isolated from rat liver nuclei stimulated chromatin-templated transcription and moreover that in the presence of the non-histone proteins qualitatively different RNA's were synthesized. One question remained unsolved : are new genes unmasked by direct binding of repressive proteins with the non-histone proteins, or does de-repression occur by some enzymatic activity of the non-histone proteins ? In the latter case phosphokinase would be the enzyme to be considered since the incubation medium contains all the compounds involved in phosphorylation.

Recently several groups have reported observations concerning protein kinases (2 - 15), which suggests a functional role of phosphorylation of the chromatin proteins in the regulation of genetic message trascription.

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In this work, we present results which suggest a correlation between the phosphokinase activity of the non-histone proteins and their ability to stimulate chromatin-templated RNA synthesis.

MATERIALS AND METHODS

The non-histone proteins and the chromatin were prepared from rat liver nuclei isolated according to Chauveau et al. (16) as previously described (1). The last step of the preparation of the non-histone proteins was performed on a DEAE-cellulose column with elution by a discontinuous NaCl concentration gradient (fig. 1).

The kinase activity was assayed by measuring the radioactivity of phosphate incorporated from ATP-γ-^{32}P into non-histone protein and into histone F_{2b}. The acid insoluble material was collected on a millipore filter and the radioactivity was measured in a Nuclear Chicago liquid scintillator. Alkali-labile phosphate bound to proteins was determined by the method of Kleinsmith et al. (2).

For assaying the template activity of the phosphorylated chromatin, chromatin was pretreated as follows : the "condensed" chromatin (17), containing 1.0 mg of DNA was incubated with 100 µmoles of Tris-HCl, pH 8.0, 100 mµmoles of ATP, 25 µmoles of $MgCl_2$, 1.0 mg of the non-histone proteins at 37° for 15 min.. The final volume was 1.0 ml. The chromatin was then washed twice with 0.02 M Tris-HCl, pH 7.5, to remove the free ATP and the non-histone proteins, and resuspended in 0.02 M Tris-HCl, pH 7.5. The DNA concentration was determined by the diphenylamine reaction (18). The template activity of the phosphorylated chromatin was assayed as previously described (1). RNA polymerase was prepared from M. lysodeikticus and purified up to the $(NH_4)_2\ SO_4$ precipitation step according to Nakamato et al. (19).

RESULTS AND DISCUSSION

Four protein peaks were obtained from the DEAE-cellulose column (fig. 1). The kinase activity was assayed for each of them (Table I). It was previously reported that the peak eluted with 0.3 M NaCl stimulated chromatin templated RNA synthesis (1). The same peak carried along the kinase activity (Table I). The proteins of this fraction were phosphorylated even without any addition of exogenous substrate. Nevertheless the addition of 100 µg of histone F_{2b} to the incubation mixture greatly increased the total incorporation of ^{32}P. The slight incorporation in the peak III protein was probably due to a contamination by the material present in peak II. The kinase activity was not stimulated by cyclic AMP. The peak III and IV were not likely to contain the cyclic AMP binding pro-

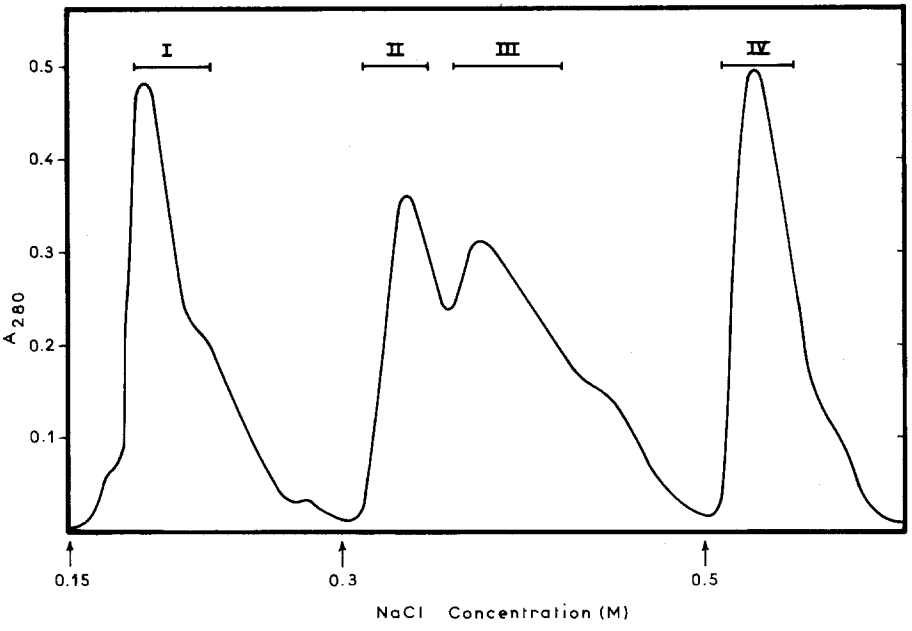

Fig. 1. DEAE-cellulose chromatography of non-histone proteins. The non-histone proteins were eluted with various NaCl concentrations in a 0.05 M Tris-HCl (pH 8.0) buffer containing 1 mM EDTA and 1 mM β-mercaptoethanol. Each peak (I-IV) was pooled and proteins were concentrated for assay.

TABLE I

Kinase activity of non-histone proteins (NHP) eluted from DEAE-cellulose column

The reaction mixture contained 20 µmoles of Tris-HCl, pH 7.5, 2,5 mµmoles of ATP-γ-^{32}P, 5 µmoles of $MgCl_2$, 25 µmoles of NaCl and 20 µg of non-histone proteins preparation. The total volume was 0.25 ml. Where indicated, 100 µg of rat liver histone F_{2b} fraction and/or $5x10^{-6}$ cyclic AMP were added. The incubation was carried out at 37° for 10 min. and was stopped by addition of 10 % trichloroacetic acid.

Protein peak	Exogeneous substrate	^{32}P-Incorporated (c.p.m.) No cAMP	$5x10^{-6}$M cAMP
I	None	338	-
II	None	2,206	2,254
III	None	813	-
IV	None	299	-
NHP	100 µg of Histone F_{2b}	368	390
I	100 µg of Histone F_{2b}	324	313
II	100 µg of Histone F_{2b}	4,270	4,377
III	100 µg of Histone F_{2b}	885	879
IV	100 µg of Histone F_{2b}	319	333
II + III	100 µg of Histone F_{2b}	4,713	4,894
II + IV	100 µg of Histone F_{2b}	4,210	4,380
II Alkali-treatment	100 µg of Histone F_{2b}	615[a]	

[a] After removal of alkali-labile phosphate.

tein reported by Kumon et al. (13), for the kinase activity of the peak II was not decreased when incubated with either the peak III or the peak IV proteins, nor activated by adding cyclic AMP to the mixture.

Most of the phosphate transfered to histone and phosphate acceptor proteins was alkali-labile.

In another set of experiments, rat liver chromatin was pre-incubated with the non-histone proteins, ATP and Mg^{2+}. The peak II

TABLE II

Phosphorylation of chromatin

In the complete system, the reaction mixture contained 10 µg of DNA in chromatin and 40 µg of non-histone proteins eluted in peak II (NHP). Incubation was carried out at 37° for 10 and 20 min.

System \ Phosphorylation	^{32}P-Incorporated (c.p.m.) 10 min.	20 min.
Chromatin alone	1,502	1,841
NHP alone	567	776
Chromatin + NHP	2,787	3,121

TABLE III

Template activity of chromatin preincubated in various conditions

The complete preincubation system contained ATP, Mg^{++} and non histone protein (NHP) in the RNA polymerase reaction mixture, chromatin contained 20 µg of DNA.

Preincubation System	mµmole ATP incorporated Exp.No. 1	2	3
Complete	0.131	0.136	0.196
Complete - NHP	0.095	0.092	0.138
Complete - ATP	0.094	0.098	0.136
Complete - NHP - ATP	0.099	0.097	0.137
Complete - ATP - Mg++	0.098	0.096	0.135
Complete - NHP - ATP - Mg++	0.104	0.104	0.145
No Preincubation	0.092	0.098	0.140

eluted from DEAE-cellulose column was used in these experiments to catalyze phosphorylation. Chromatin itself was phosphorylated by endogeneous protein kinase, since it contained non-histone proteins as one of its components. The phosphorylation was, however, increased more than additional value by addition of the exogeneous non-histone proteins (Table II).

The increase of the chromatin template activity by phosphorylation is shown in Table III. The template activity of chromatin for RNA polymerase reaction was increased only by the complete system of preincubation, which indicated that there was a correlation between the non-histone protein kinase activity and the activation of chromatin as a template of transcription. The rates of RNA syntheses in the presence of chromatins preincubated with and without ATP were compared (fig. 2).

Recently, Takeda et al. (15) reported that two protein kinases were associated with rat liver chromatin and catalyzed the phosphorylation of nuclear non-histone proteins. Furthermore, the nuclear phosphoproteins prepared by the method of Langan contained kinase

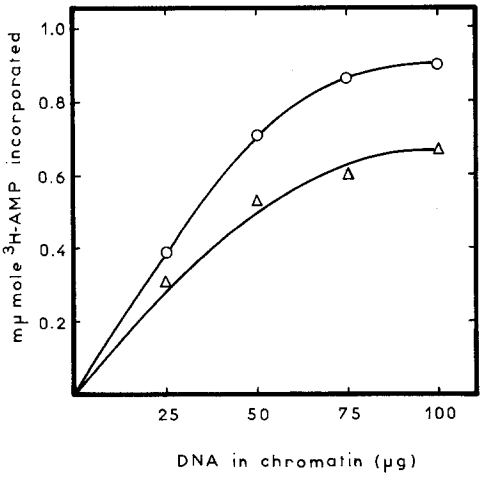

Fig. 2. Comparison of the template activity of chromatin samples preincubated with (——o——) and without (——Δ——) ATP.

activity and were phosphorylated without any addition of exogeneous enzyme (3)(9).

In general, protein kinase has a broad substrate specificity, and so there is no evidence that phosphoprotein in the non-histone proteins is an intermediate of phosphate transfer to histones.

Martelo et al. (20) reported that protein kinase from rabbit skeletal muscle and rabbit reticulocyte stimulated the DNA-dependent RNA polymerase of E.coli with T4 DNA as template and additional stimulation of RNA synthesis occurred in the presence of 3',5'-AMP. The non-histone proteins employed in this study did not stimulate native DNA-templated transcription using RNA polymerase from M. lysodeikticus (1).

Considering the present results, it can be concluded that the protein kinase associated with rat liver nuclei is involved in the control of gene de-repression. Further purification and fractionation of the non-histone proteins and their role on the mechanisms of gene expression are under investigations.

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