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The effect of cycloheximide on nuclear uH2A content[1]

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Summary. The effect of intraperitonal cycloheximide administration on acid-soluble rat liver chromatin proteins has been investigated by electrophoresis in acetic acid-urea polyacrylamide gels. A nonhistone protein, which migrates between oxidized histone H3 and histone H1 has been found to be increased in amount following cycloheximide treatment. This protein seems to be identical with semihistone protein H24 (uH2A). A possible relationship of uH2A to the inhibition of rRNA synthesis is discussed.
Key words. Rat liver; liver, rat; chromatin, rat liver; cycloheximide; nonhistone protein.

Histones and their post-synthetically modified forms are implicated, like nonhistone proteins, in the regulation of gene expression. Semihistone protein A24 (uH2A)[3] initially aroused interest because of the observation that its content in rat liver nucleoli decreases during nuclear hyperfunction (liver regeneration) induced by partial hepatectomy or thioacetamide administration[4]. This is particularly noteworthy since chromatin activation[5,6] is correlated with the cleavage of uH2A into histone H2A and ubiquitin[7]. Purified protein uH2A has been shown to have a branched structure[8] consisting of ubiquitin[8–11] and histone H2A, the carboxyl terminus of ubiquitin being attached to the ε-amino group of the lysine residue 119 of histone H2A[12]. uH2A appears to be an integral component of the nucleosomes replacing histone H2A in the nucleosome histone core[13,14]. There are different and contrasting suggestions on the role of uH2A which range from inhibition to activation of transcription[6,15,16]. In order to obtain further insight into the biological function of uH2A, we investigated the changes in its amount resulting from treatment of rats with cycloheximide. Our results suggest that cycloheximide (which has previously been shown to enhance, at a concentration fully inhibitory for protein synthesis, the activities of RNA polymerases II and III[17]) induces an increase in the amount of uH2A.

Materials and methods. Preparation and fractionation of chromatin. Minced rat liver was homogenized, using an Elvehjem-Potter homogenizer, in 5 mM $MgCl_2$, 25 mM KCl, 50 mM Tris-HCl, pH 7.4, 250 mM sucrose and 0.5% triton X-100. After centrifugation at 1500 × g for 15 min the nuclear pellet obtained was suspended and centrifuged at 2000 × g in the same buffer containing 1.6 M sucrose. The nuclei were collected and resuspended by stirring in 80 mM NaCl and 20 mM Na-EDTA pH 7.4 and then centrifuged at 10,000 × g for 15 min. This step was repeated. Then the chromatin was washed twice in 80 mM NaCl, 10 mM Tris-HCl pH 7.4 and twice in 10 mM Tris-HCl pH 7.4 and stored at −65 °C until use. All steps were carried out in presence of 0.1 mM phenylmethylsufonylfluoride (PMSF). The chromatin fraction containing histones and uH2A was extracted with 0.2 M H_2SO_4 and precipitated by making the solution 80% in ethanol. The precipitate was collected by centrifugation at 10,000 × g for 15 min, washed twice in ethanol and dried under vacuum.

Electrophoretic procedure. 0.2 M H_2SO_4 soluble chromatin proteins were analyzed using acetic acid-urea polyacrylamide gel electrophoresis[18]. The gels were stained with Amido Black and scanned using a CAMAG Electrophoresis Scanner.

Results. Histones and acid-soluble nonhistone proteins were extracted from equal amounts of rat liver chromatin samples and analyzed by acetic acid-urea gel electrophoresis. Figure 1 shows the densitometric analysis of proteins on acetic acid-urea polyacrylamide gels. After cycloheximide treatment (10 mg/kg i.p. , 30–45 min before decapitation) an increase in the amount of the protein which migrates between histone H1 and oxidized histone H3 was observed. Cyclophosphamide treatment (40 mg/kg, i.v., for three consecutive days) did not induce a significant change in its nuclear amount (data not shown). The same protein was found to decrease after the treatment with thioacetamide (50 mg/kg, i.p., for three consecutive days). These findings, which are summarized in the table, suggested a possible identity with uH2A[19]. This protein was later purified by preparative acetic acid-urea electrophoresis (fig. 2) and was observed to have an electrophoretic mobility a little higher than histone H1 on SDS-polyacrylamide gels[20] (fig. 3). Its identity with uH2A was further supported by NH_2-terminal analysis (data not shown).

Densitometric tracings of Amido Black stained acetic acid-urea polyacrylamide gel electrophoregrams of 0.2 M H_2SO_4 soluble chromatin proteins (fig. 1) revealed also a slight increase in the content of oxidized histone H3 and of histone H4 in thioacetamide-treated rats.

Discussion. The results of this study show an increase in the amount of uH2A after cycloheximide treatment. Cycloheximide, a drug frequently used for studying the mechanisms of gene expression, is characterized by its dual effect on transcrip-

The effect of drug treatment on nuclear uH2A content

Treatment	Ratio uH2A/core histones	Control (%)
Control	0.038	100
Cycloheximide	0.056	147
Thioacetamide	0.021	55

Both drugs were employed i.p.; thioacetamide (50 mg/kg) for three consecutive days, cycloheximide (10 mg/kg) 30–45 min before decapitation. Acid-soluble rat liver chromatin proteins were extracted with 0.2 M H_2SO_4 and analyzed as described in the legend to figure 1. The ratios of protein uH2A to core histones (averages of 2 determinations) computed by weighing the areas under the protein peaks represent the computerized evaluation of the data given in figure 1. Data from repeated experiments – not included in the above evaluation – have shown unequivocally the cycloheximide-promoted increase of uH2A content.

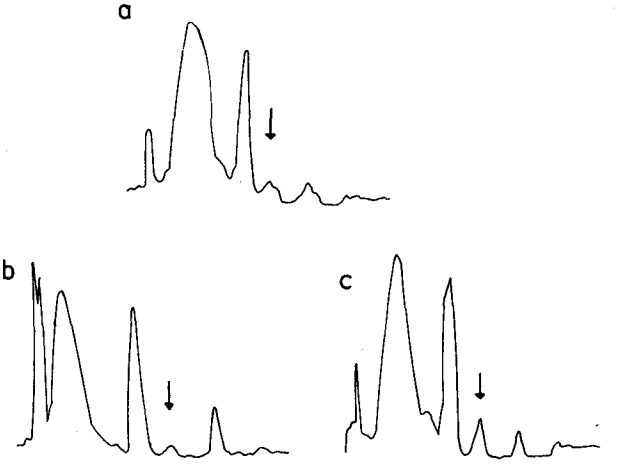

Figure 1. Densitometric tracing of 0.2 M H_2SO_4 soluble chromatin proteins electrophoresed in acetic acid-urea polyacrylamide gels. Electrophoresis and the staining of the gel discs with Amido Black were done as by Goldknopf et al.[20]. 0.2 M H_2SO_4 soluble chromatin proteins from *a* normal rat liver, *b* liver of thioacetamide treated rats (50 mg/kg, i.p., consecutively for three days), *c* liver of cycloheximide treated rats (10 mg/kg, i.p., 30–45 min before decapitation). Arrows indicate protein uH2A.

tional activities in the nucleus. Aside from an enhancing effect on the extent of RNA synthesis, probably by stimulating the activities of RNA polymerases II and III[17-21], cycloheximide causes an impairment of nucleolar transcription[22-25]. A time-dependent decrease in the activity of RNA polymerase I observed after cycloheximide treatment[17] may account for the decrease in the nucleolar activity accompanied at the same time by partial condensation of the nucleolar structure[26].

As indicated above, the content of the protein uH2A reveals a reciprocal relationship to different states of activity of the nucleolus[4,27]; a decrease in its amount during nucleolar hypertrophy is correlated with the activities of ribosomal RNA genes[4]. Thus the increase in the amount of uH2A after cycloheximide treatment may be involved in the depression of nucleolar transcription.

Cycloheximide-promoted effects on the uH2A content may be explained in terms of the inhibition of A24 lyase[7], the enzyme which cleaves protein uH2A. The first observed increase in protein uH2A after treatment of Ehrlich Ascites Tumor Cells with 1-methyl-1-nitrosourea (MNU) and 1,3-Bis (2-chloroethyl)-1-nitrosourea (BCNU) been correspondingly interpreted by the inhibition of the cleavage of uH2A by these alkylating agents[28]. However, for the present, other explanations also remain possible.

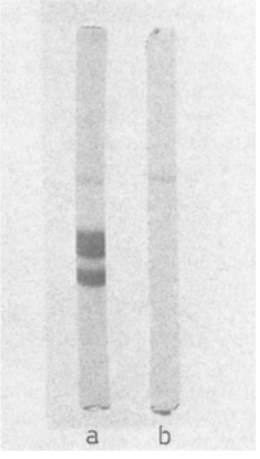

Figure 2. Analysis of protein uH2A on acetic acid-urea-polyacrylamide gels. *a* 0.2 M H_2SO_4 extract of 5% $HClO_4$ extracted rat liver chromatin, *b* purified protein uH2A.

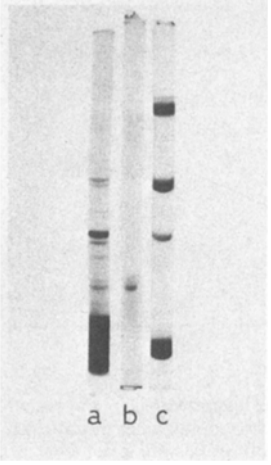

Figure 3. Electrophoretic analysis of protein uH2A on SDS-polyacrylamide gels. *a* 100,000 × g pellet profile of 0.5 M NaCl soluble chromatin proteins, *b* protein uH2A, *c* standard proteins (bovine serum albumin, ovalbumin, chymotripsinogen, cytochrome-C).

The role of uH2A in the control of gene activities is not yet understood and the reports on this matter are, moreover, not consistent. uH2A does not appear to promote conformational changes in nucleosomes in which it replaces histone H2A[5,13,29]. It has been proposed that uH2A acts mainly at the 'supranucleosomal' level[11,13]. On the other hand the role of ubiquitin in cytoplasmic ATP-dependent proteolysis[30] suggests also a similar role for uH2A. The rapid turnover of this protein may cause conformational changes in chromatin. Investigation of ATP-dependent proteolysis of uH2A during chromatin activation may bring further insight into the mechanisms involved.

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