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FUNCTIONAL HETEROGENEITY OF CHROMATIN FRACTIONS OBTAINED BY LIMITED HYDROLYSIS OF RAT LIVER NUCLEI BY ENDOGENOUS Ca^{2+},Mg^{2+}-DEPENDENT ENDONUCLEASE

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KEY WORDS: endogenous nuclease; chromatin; transcription; repair.

Fragmentation of chromatin by nucleases is a standard technique which, since the beginning of the 1970s, has achieved widespread popularity for the study of chromatin structure and function [3]. Knowledge of the enzymologic characteristics of the commercial nucleases usually used for this purpose and analysis of the hydrolysis products provide definite ideas on the structural-functional specificity of the chromatin regions which may form fragments. Previously the writers have used endogenous nuclease from rat liver nuclei as the principal enzyme [2, 5, 6]. In this case the use of a nuclease which participates directly in the processes of genome function is particularly interesting [2]. Unfortunately, however, there are no unambiguous data on the functional loads of this enzyme in the literature. As an approach to the study of this problem the writers decided to investigate the functional specificity of chromatin fractions differing in accessibility for endogenous nuclease in the initial stages of hydrolysis (1-3% acid-soluble DNA). Such an approach is justified because, according to our own data [2, 7] and data in the literature [13], the regions into which chromatin is fragmented by endogenous nuclease in the initial stages of hydrolysis are those very regions with which molecules of the enzyme are associated.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats. The method of isolation of the liver nuclei and conditions for activation of endogenous nuclease were described previously [8]. The nuclei were incubated at 37°C and the concentration of nuclear DNA was 6 mg/ml. To fractionate the hydrolyzed chromatin 20 volumes of 0.5 mM EDTA-Na_2, pH 7.0, were added and the suspension was stirred for 30 min on ice. Samples were divided into solubilized (S_1) and nonsolubilized (P_1) fractions by centrifugation at 12,000g (15 min). $MgCl_2$ was added to S_1 up to 2 mM and, after incubation for 30 min on ice, it was centrifuged at 12,000g (15 min). The supernatant fraction was called S_2 and the residue P_2. Fraction S_2 was lyophilized for electrophoretic investigation. Preparation of the DNA samples and the conditions of electrophoresis were described previously [8]. To investigate DNA synthesis, [^{14}C]thymidine was injected into the animals' portal vein 5 min before decapitation in a dose of 100 μCi/100 g. To investigate RNA synthesis, [^{3}H]orotic acid was injected intraperitoneally into the rats 5 min before decapitation in a dose of 30 μCi/100 g. Regeneration of the liver was stimulated by removal of 70% of the organ. Hydrocortisone (from Gedeon Richter, Hungary) was injected intraperitoneally in a dose of 5 mg/100 g 6 h before decapitation. Separation and quantitative determination of DNA and RNA and measurement of radioactivity were carried out as described previously [5].

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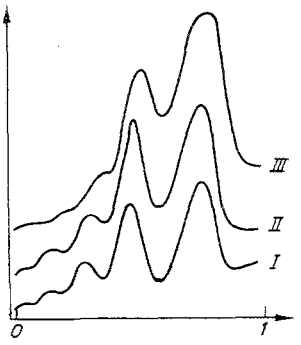

Fig. 1

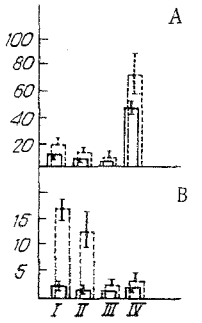

Fig. 2

Fig. 1. Densitograms of DNA of fractions S_2. I) 5 min, II) 15 min, III) 30 min of incubation. Abscissa, mobility (in relative units); ordinate, optical density (in relative units).

Fig. 2. Distribution of fast-labeled RNA (A) and DNA (B) in chromatin fractions. A: Continuous line — control, broken line — 6 h after injection of hydrocortisone; B: continuous line — intact liver, broken line — regenerating liver (24 h of regeneration). Quantity of label in RNA, expressed per milligram DNA, taken as the RNA/DNA ratio. Horizontal axis: I) initial chromatin, II) fraction P_1, III) fraction P_2, IV) fraction S_2; vertical axis: A) RNA/DNA ratio (in cpm·10^{-3}/mg DNA), B) radioactivity of DNA (in cpm/mg·10^{-3}).

EXPERIMENTAL RESULTS

The results of electrophoresis of DNA of fraction S_2 are given in Fig. 1. They show that after incubation for 5 min the main components of this fraction were mono- and dinucleosomes (although discrete fragments measuring 1000-1200 nucleotide pairs were detected). Much of the material in the total hydrolysates, i.e., in preparations of unfractionated chromatin, at this stage consists of high-molecular-weight DNA fragments of not less than 60 megadaltons. Analysis of denatured DNA preparations in 12% polyacrylamide gel in the presence of 7 M urea showed that S_2 nucleosomes are degraded to a greater degree by endogenous nuclease than nucleosomes in the other fractions. The S_2 fraction thus contains chromatin fragments capable of being excised and fragmented by endogenous nuclease at a faster rate than chromatin itself is broken down by the action of this enzyme. On incubation of the nuclei for 5 to 30 min the content of the S_2 fraction rose from 1 to 11% of the total chromatin; the acid-soluble DNA under these circumstances did not exceed 3% of the total. Consequently, the S_2 fraction obtained under these conditions contains monosomes and their oligomers, products of the initial stages of autolysis of chromatin. The writers showed previously that this fraction is richer in fast-labeled RNA than unfractionated chromatin [2]. It might be supposed that if this fast-labeled RNA reflects the transcription activity of this particular fraction, its content will rise, through the action of agents activating the genome, faster than the content of fast-labeled RNA in the remaining fractions. To test this hypothesis the effect of hydrocortisone on distribution of fast-labeled RNA was investigated in the chromatin fractions (Fig. 2A).

Clearly induction by hydrocortisone increased the content of fast-labeled RNA in total chromatin by 27% compared with the control, and in the S_2 fraction by 72% compared with the control. This is evidence of the origin of the S_2 fraction from transcribed regions of chromatin and, considering the conditions under which it was obtained, binding of endogenous nuclear nuclease with these regions. To probe the specific character of autolysis of chromatin further, the distribution of fast-labeled DNA was studied in the chromatin fractions in regenerating (24 h of regeneration) and intact liver (Fig. 2B). The specific radioactivity of DNA in fraction S_2 in resting liver was higher than the specific radioactivity of DNA in other fractions (although it did not differ from the original chromatin, most probably because of cleavage of part of the fast-labeled DNA to acid-soluble products). The picture was sharply

TABLE 1. Time Course of Specific Radioactivity of DNA in Fraction during Cleavage of Chromatin by Endogenous Nuclease

Quantity of DNA in fraction S_2, % of total DNA of chromatin	Specific radioactivity of DNA in fraction S_2, % of initial level
3,7	100
7,2	83,3±5,3
11,0	66,6±3,3

Legend. Specific radioactivity when DNA content in fraction is 3.7% of total, corresponding to 5 min of autolysis of chromatin under standard conditions, taken as initial level (100%) of specific radioactivity of fraction S_2.

altered in the regenerating liver and the overwhelming majority of fast-labeled DNA was concentrated in the P_1 fraction, i.e., that most resistant to the action of endogenous nuclease. Comparison of Figs. 2A and 2B clearly reveals functional heterogeneity of the chromatin fractions and the "attraction" of endogenous nuclease to regions capable of transcription though not of replication. This gives rise to a certain paradox, due to the fact that existing experimental data indicate a role for this enzyme in systems of DNA synthesis [9, 10]. The substantial differences between the distribution of fast-labeled DNA in chromatin fractions from regenerating and intact liver are noteworthy in this connection. For a more detailed verification of the sensitivity of DNA newly synthesized in the resting liver to endogenous nuclease, specific radioactivity of DNA was studied in the S_2 fraction during prolongation of chromatin autolysis (Table 1). The main conclusion from these results is that during nonreplicative DNA synthesis the S_2 fraction in the initial stages of hydrolysis and, consequently, regions of chromatin exposed to primary attack by endogenous nuclease (or associated with the enzyme) are enriched by newly synthesized DNA chains.

Analysis of these data in conjuction with the results of the present investigation to study the action of endogenous nuclease on chromatin methylated *in vitro* [2, 5, 6] led to the suggestion that DNA-synthesizing systems, in whose function endogenous Ca^{2+},Mg^{2+}-dependent endonuclease of rat liver nuclei is involved, are DNA repair systems [7].

This could explain the mechanism of "demethylation" of DNA during activation of endogenous nuclease as an alternative to specific enzymic demethylation [1]. This could also explain the "attraction" of endogenous nuclease to the transcribed regions, for arguments exist in support of differential repair, linking the intensity of repair of particular regions of the genome with the intensity of their transcription [12].

Data indicating involvement of Ca^{2+},Mg^{2+}-dependent rat liver nuclear nuclease in repair processes also have been obtained recently [11, 14, 15]; in particular, its binding with poly-(ADP-ribose) inhibits the enzyme and, at the same time, reduces the velocity of unplanned DNA synthesis [14, 15].

It thus seems most likely that endogenous Ca^{2+},Mg^{2+}-dependent nuclease is one of the components of repair systems in eukaryotes, i.e., of systems responsible for the genetic stability of the cell [4].

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EFFECT OF CORTISOL ON REPARATIVE SYNTHESIS AND METHYLATION OF RAT LIVER DNA

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KEY WORDS: cortisol; reparative synthesis; methylation of DNA.

Corticosteroid hormones are known to inhibit total DNA synthesis [9], to affect methylation of DNA [12], to activate transcription [6], and to increase the fraction of active chromatin [10] in rat liver cells. Against the background of general inhibition, some degree of activation of DNA synthesis is observed, coinciding in time with activation of transcription [6]. Amplification of the transcribed genes is possible under these circumstances [4], evidence for which is given by a change in the primary structure of DNA under the influence of the corticosteroid analog dexamethasone [3]. It has recently been suggested that demethylation of DNA by excision of 5-methylcytosine (MC), and also activation of nuclease, correlating with gene expression, can induce DNA injuries [1]. As a result of these injuries corresponding activation of reparative DNA synthesis may arise.

The object of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used and the experiments were done in April. Cortisol (Richter, Hungary) was injected intraperitoneally in a dose of 2 mg/100 g. To assess reparative DNA synthesis, hydroxyurea, which inhibits replicative DNA snythesis, was injected into the animals 1 h before sacrifice in a dose of 50 mg/100 g, and this was followed 10 min later by injection of [^{3}H]thymidine (55 Ci/mmole, USSR) in a dose of 100 μCi/100 g [5]. The animals were killed by decapitation. The freshly removed liver was quickly cooled on ice and homogenized in a Dounce homogenizer at 0°C in a solution containing 0.15 M NaCl, 0.1 M EDTA, pH 8.0, an equal volume of 1 N NaOH was added, and the mixture was allowed to stand for 12 h at 37°C, after which treatment of the material followed the usual lines [11]. The acid-insoluble residue was collected on membrane filters, washed with ethanol, and transferred to scintillation cuvettes. The filters were covered with 1 ml methylcellosolve and, a few hours later (after they had dissolved) 9 ml of toluene scintillator (4 g PPO + 400 mg POPOP to 1 liter toluene) was added and radioactivity was measured on a liquid scintillation counter.

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