PHYSICOCHEMICAL PROPERTIES OF CHROMATIN FRACTIONS OF CELL NUCLEI IN THE NORMAL AND REGENERATING RAT LIVER

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UDC 612.35+612.35:612.6.03].014.24

A comparative study was made of the composition and physicochemical properties of the condensed and diffuse chromatin isolated from cell nuclei from the normal rat liver and from the regenerating liver 20 and 32 h after partial (⅔) hepatectomy. The results showed that after 20 h the RNA concentration in the diffuse chromatin was increased by 35%, returning to normal after 32 h. The RNA concentration in the condensed chromatin was unchanged. The protein concentration in the diffuse chromatin was reduced by 20% after 20 h, returning close to normal after 32 h. The protein concentration in the condensed chromatin was unchanged. By the 20th hour of regeneration the compactness of arrangement of the macromolecules in the diffuse chromatin was considerably reduced, while in the condensed chromatin it was almost unchanged. It is concluded that the DNA in diffuse chromatin possesses greater stereochemical accessibility at the 20th hour of regeneration.

An important index of regeneration of organs in higher animals is the temporary sharp activation of template activity of the chromatin of the cell nuclei which precedes active mitotic cell division[4]. An essential prelude to the synthesis of new RNAs on previously repressed gene loci, along with other factors, is modification of the structure of the chromatin, but no experimental evidence is available on this question. The nuclear chromatin can exist in two states: condensed (heterochromatin) and diffuse (euchromatin). These types of chromatin differ both in their structure and in their activity of RNA synthesis (the diffuse form is much more active) [1, 2, 6].

In the investigation described below an attempt was made for the first time to compare the composition and physicochemical properties of these fractions, which reflect their connection with the change in genetic activity of the cells during regeneration of the rat liver after partial (⅔) hepatectomy.

EXPERIMENTAL METHOD

Noninbred male rats weighing 160-180 g were used in the experiments. In the experimental animals two-thirds of the liver was removed by the method of Higgins and Anderson [7], and the animals were decapitated 20 and 32 h later. These times were chosen as the mean maximum of DNA synthesis (20 h) and the maximum of mitotic activity (32 h). The mean mitotic coefficient determined at these times was 0.03 and 1.95%. A mock operation was performed on the control rats which were sacrificed at the same times as the experimental animals. All manipulations were carried out in a cold room at +2°C.

Nuclei were isolated from the liver cells of the control and experimental animals by Chauveau's method [5], after which whole chromatin and its fractions (condensed and diffuse) were obtained from the

Laboratory of Molecular Biology, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 2, pp. 38-40, February, 1973. Original article submitted February 18, 1972.

TABLE 1. Concentrations of Nucleic Acids and Protein in Chromatin and Its Fractions Isolated from Cell Nuclei of Normal Rat Liver and of Liver Regenerating 20 and 32 h after Partial Hepatectomy

Chromatin	State of liver	Time after operation (in h)	DNA (in mg/ml)	RNA (in % of DNA)	Protein (in mg/ml)	Protein DNA
Whole	Normal		1,03	9,9	2,33	2,26
	Regeneration	20	0,75	14,2	1,63	2,17
		32	0,83	11,5	1,98	2,38
Condensed	Normal		1,37	6,0	3,19	2,32
	Regeneration	20	1,07	5,6	2,52	2,35
		32	1,01	5,9	2,27	2,24
Diffuse	Normal		0,62	17,0	1,66	2,67
	Regeneration	20	0,70	23,0	1,48	2,11
		32	0,64	19,5	1,65	2,57

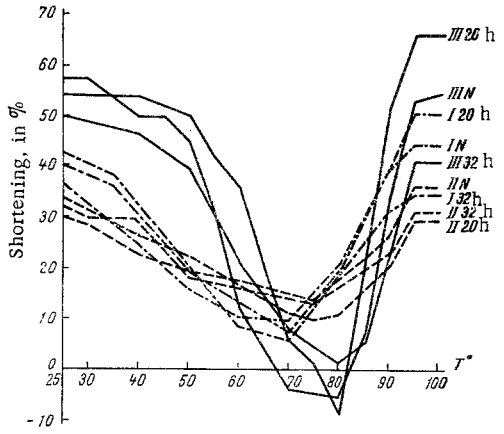

Fig. 1

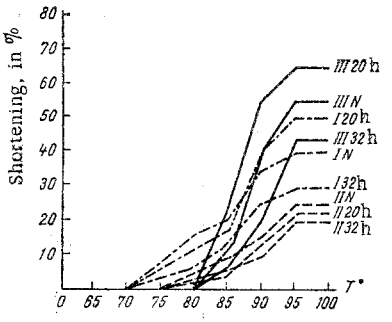

Fig. 2

Fig. 1. Relative length of fibers of chromatin and its fractions isolated from nuclei of normal and regenerating rat liver as a function of temperature. Here and in Fig. 2: I) whole chromatin; II) condensed chromatin; III) diffuse chromatin; N) from normal liver; 20 h) from regenerating liver 20 h after partial hepatectomy; 32 h) from regenerating liver 32 h after hepatectomy.

Fig. 2. Curves of structural changes in fibers of chromatin and its fractions isolated from nuclei of the normal and regenerating rat liver in the region of the "helix-coil" transition of DNA.

nuclei by Frenster's method [6]. All the fractions were dissolved in 0.7 M NaCl and the DNA and RNA concentrations in them were determined spectrophotometrically after separation by the method of Schmidt and Thannhauser [9]. The protein concentration was determined by Lowry's method [8].

To investigate the physicochemical parameters of the chromatin and its fractions isolated from the liver at different times after partial hepatectomy, a thermomechanical method was used [3]. In this way it is possible to obtain information on the helix-coil transition of DNA molecules in the chromatin and on the character of interaction between the DNA and protein molecules in the supramolecular structure in a medium of physiological ionic strength.

EXPERIMENTAL RESULTS

The results of a study of the chemical composition of whole chromatin and its fractions from the normal liver and 20 and 32 h after partial hepatectomy during regeneration are given in Table 1.

Analysis of the results given in Table 1 shows that in the normal liver the RNA concentration (as a percentage of DNA) was highest of all in the diffuse chromatin, and that after regeneration for 20 h the RNA concentration in the diffuse chromatin was 35% higher than normal, and it returned close to normal after 32 h.

The RNA concentration in condensed chromatin showed virtually no change in the course of regeneration. The protein concentration relative to DNA in the normal liver was highest in diffuse chromatin. After 20 h the protein concentration in the diffuse chromatin was reduced by 20%, and it returned close to normal after 32 h. In the condensed chromatin the protein concentration relative to DNA was almost unchanged.

Investigation of the supramolecular systems of chromatin and its fractions during regeneration by a thermomechanical method provided for the first time data on the physicochemical properties of condensed and diffuse chromatin in a medium of physiological ionic strength. As the curves given in Figs. 1 and 2 show, during regeneration the compactness of arrangement (supercoiling, possibly) of the macromolecules in the diffuse chromatin system 20 h after the operation was considerably reduced, as shown by an increase in amplitude of the melting of this fraction from 55% (normal) to 67%. By 32 h after the operation the amplitude of melting of the diffuse chromatin was reduced to 42%, indicating a more compact arrangement of the diffuse chromatin even than under normal conditions. The condensed chromatin changed only slightly during regeneration, as reflected in a very slight tendency toward supercoiling 32 h after the operation.

It can be concluded from these results that significant structural changes take place in the diffuse chromatin fraction during regeneration, as reflected in a change in the functional activity of the chromatin. This effect is due in all probability to the greater stereochemical accessibility of the DNA in the structurally modified diffuse chromatin, and in addition to changes in the concentration and quality of the proteins bound with the DNA, it may probably be under the control of condensation processes.

LITERATURE CITED

1. L. N. Anan'eva and A. E. Berman, Dokl. Akad. Nauk SSSR, 186, 222 (1969).
2. G. S. Arbuzova, I. M. Gryaznova, G. M. Morozova, et al., Molekul. Biol., 2, 303 (1968).
3. D. M. Spitkovskii, The Radiosensitivity of the Deoxyribonucleoproteins of Somatic Cells as a Function of Their Structural Organization. Doctoral Dissertation, Moscow (1969).
4. N. L. R. Bucher and M. N. Swaffield, Biochim. Biophys. Acta, 108, 551 (1966).
5. J. Chauveau, G. Moule, and R. Roiller, Exp. Cell Res., 11, 317 (1956).
6. J. H. Frenster, V. G. Allfrey, and A. E. Mirsky, Proc. Nat. Acad. Sci. (Washington), 50, 1026 (1963).
7. G. M. Higgins and R. M. Anderson, Arch. Path., 12, 186 (1931).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
9. G. Schmidt and S. J. Thannhauser, J. Biol. Chem., 161, 83 (1945).