Spectrum of Histone Proteins and Acridine Orange Binding by Liver Cell Chromatin in Rats at Different Times after Partial Hepatectomy

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Changes in the spectrum of hepatic histones characteristic of active chromatin were observed in rats as early as 3 h after partial hepatectomy. At 6-9 h postsurgery, acridine orange binding to deproteinized chromatin areas was considerably increased. At 13 h the histone spectrum of liver cells from operated rats did not differ from that of control samples from sham-operated animals.

Key Words: *partial hepatectomy; histones; chromatin activation; rat liver*

Tissue regeneration continues to be a subject attracting the attention of investigators. Animal models of tissue regeneration following partial hepatectomy (PHE) are therefore of considerable interest. Hepatocytes are highly differentiated polyfunctional cells, and partial removal of the liver provides a powerful stimulus for their proliferation. It remains, however, unknown how the proliferative process is triggered and how it proceeds. What is clear is that it involves much of the liver's genome with strong chromatin activation and intensified expression of many genes, which eventually results in the regeneration of this organ and the restoration of its functions. There is evidence that an important role in the regeneration is played by cellular lysosomal enzymes which enhance the limited proteolysis of chromosomal proteins, primarily histones [3], but the contribution made by the lysosomal apparatus of cells to chromatin activation needs further study. The aim of the present investigation was to examine changes in histone proteins and chromatin activation at different times after PHE.

MATERIALS AND METHODS

Wistar rats weighing 150-200 g were used. PHE was performed as described [8], with the removal of two-thirds of the liver (the right lobe) under ether anesthesia. At 3, 6, 9, and 13 h after the operation, rats were killed by decapitation and liver tissue samples were collected. Control samples were taken from the livers of sham-operated rats.

Liver cell nuclei were isolated and purified according to Blobel and Potter [5]. Histones from the purified nuclear fraction were isolated by extraction in 0.5 N sulfuric acid and then centrifuged for 20 min at 18,000 rpm in a Beckman L5-75 centrifuge (SW-41Ti rotor). Histone proteins contained in the supernatant were precipitated in 6 volumes of ice-cold acetone by further centrifugation for 20 min at 2500 rpm.

For a study of histone spectra, electrophoresis was performed by the method of Panyim and Chalkley [10] with modifications [6,13], using 0.9 M acetic acid as the electrode buffer. The gel was stained with a 0.2% Coomassie blue R-250 solution. The electrophoregrams were subjected to densitometry in a microphotometer. The areas of the peaks produced by individual histone fractions were read by a Sever computer at the Institute of Automation and Electrometry, Siberian Division of the Russian Academy of Sciences.

The degree of chromatin activation was estimated by titrating suspensions of purified nuclei with solutions of the fluorescent dye acridine orange (AO) at various concentrations. Staining was

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continued for 12 h at 4°C in a buffer consisting of 0.14 M NaCl, 0.02 M Na_2HPO_4, and 0.01 M citric acid, pH 4.4. The concentration of AO varied from 0.1 to 1.3 μM when the nuclei were titrated with this dye (probe) and 0.03 μM when, conversely, the probe was titrated with the nuclei. The concentration of nuclear material was 0.04 mg protein/ml for titration with the probe and varied from 0.01 to 0.08 mg/ml for titration with the nuclei. The data obtained in titrating the nuclei with the probe and vice versa were plotted in double inverse coordinates and the number of moles of the probe's binding sites was calculated per mg protein in 1 ml of the suspension.

AO is known to be incorporated between nitrogen base pairs in histone-free areas of DNA, so that its incorporation has been used as an integral indicator of deproteinization and chromatin activation [3,4].

RESULTS

The interaction between histones and DNA interferes with transcription and translation [9]. Weakening of this interaction because of limited proteolysis and various chemical modifications undergone by the histones leads to chromatin activation and enhanced gene expression. We hypothesized that these mechanisms of chromatin activation play an important part in restoring the liver mass following PHE. Data reported in the literature indicate that changes in the spectrum of histone proteins should be expected to take place during the early prereplication period [11]. Indeed, changes characteristic of active chromatin were found in the histone spectrum as early as 3 h after PHE, but they were confined almost exclusively to the histone H4. The content of its total fraction in the hepatic cell nuclei from hepatectomized rats was significantly higher than in those from sham-operated controls (Table 1), chiefly as a result of increases in the diacetylated forms of this histone (Fig. 1).

No appreciable differences were detected between the test and control groups in the spectra of other histones. The sham operation, which was also performed under ether anesthesia, appears to have been in itself a stressor strong enough to trigger the same mechanisms of chromatin activation, at least initially, as the PHE.

DNA synthesis has been reported to take place largely between 12 h and 18 h after PHE [2], while histone synthesis has been variously reported to precede DNA synthesis by 1 h or to coincide with it [7,12]. In the present study, the histone

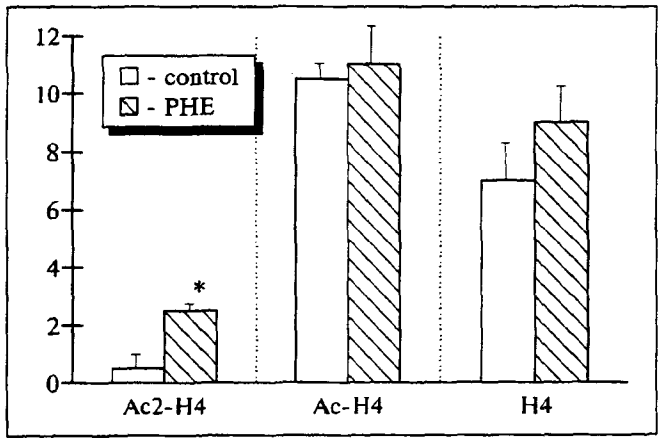

Fig. 1. Content of acetylated forms of histone H4 in rat liver 3 h after PHE, expressed in % of the total histone content. Ac2−H4 = diacetylated forms of H4; Ac−H4 = monoacetylated forms of H4. *$p<0.001$ in comparison with control samples.

spectra in hepatectomized rats at 13 h did not differ from those in sham-operated controls (Table 1 and Fig. 2). By that time most of the histone synthesis had probably been already completed so that the histones were in equimolar ratios characteristic of nucleosomes which are produced with the participation of the newly synthesized DNA. It is also possible that the S phase of the cell cycle had already been completed by that time and the cells had entered the G_2 (premitotic) phase, at least as far as the synthesis of chromatin histones is concerned.

At 13 h postoperation the level of histone H1 proteolytic products was lower than at 3 h in both hepatectomized and sham-operated rats; the content of histone H3 was also lower, whereas that of the total H2A+H2B fraction was 1.9-fold higher (Table 1). No differences between the levels of proteolytic products were detected for this latter fraction. At 13 h the content of histone H4 and particularly

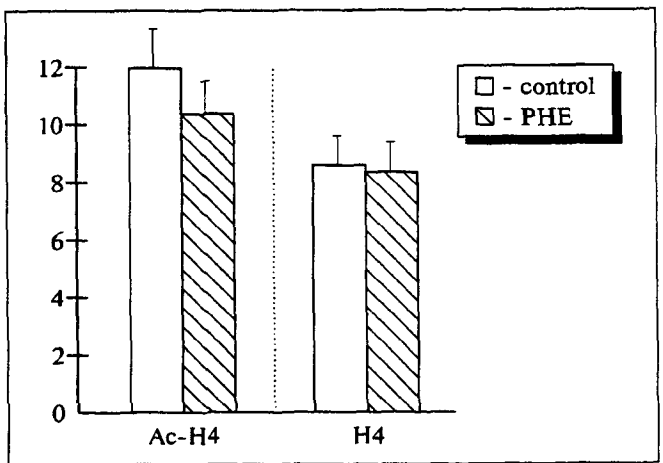

Fig. 2. Content of acetylated and nonacetylated forms of histone H4 in rat liver 13 h after PHE, expressed in % of the total histone content. Same designations as in Fig. 1.

Table 1. Proportions (%) of Histone Fractions from Rat Liver after PHE ($M \pm m$)

Histone fraction	3 h after		13 h after	
	sham operation (n=3)	PHE (n=3)	sham operation (n=5)	PHE (n=7)
H1	18.29±0.90	17.07±0.38	17.04±0.45	18.42±0.74
H1 proteolysis products	5.55±0.91	6.15±0.42	3.17±0.20*++	2.85±0.28*++
H3	29.71±0.33	26.32±1.48	16.49±0.66**	18.11±0.65++
H2A + H2B	21.98±1.46	21.71±1.40	40.25±0.94**	40.09±0.66++
H2A+H2B proteolysis products	3.34±0.29	2.97±0.37	2.75±0.39	2.17±0.18
Total H4	18.10±1.06	22.86±1.18*	20.12±0.28	18.41±0.99+

Note: n = number of rats. *$p<0.05$, **$p<0.001$ in comparison with samples taken 3 h after sham operation; +$p<0.05$, ++$p<0.001$ in comparison with samples taken 3 h after PHE.

that of its acetylated forms was significantly lower than at 3 h after PHE (Table 1).

All these data suggest that 13 h postoperation liver cell chromatin is already in a period of relative repose and its matrix activity is low in comparison with that at 3 h after PHE. These findings are consistent with data on AO binding to DNA.

Mitoses in the liver are at their peak 22 h after PHE. We measured AO binding to DNA at 6 and 9 h after PHE, i.e., during the prereplication period when chromatin is activated, which is manifested in greatly enhanced matrix activity and RNA synthesis.

As already noted, AO binds only to protein-free DNA areas by insertion between nitrogen base pairs. In this study, AO binding to DNA at 6 and 9 h after PHE exceeded twofold that after the sham operation (10.96±0.83 *vs.* 5.34±0.57 μmol/g protein). In our view, this increased binding together with the above-mentioned changes in histones reflects the unmasking of DNA from chromosomal proteins with a resultant chromatin activation. The sharp rise in the efficacy of AO binding to chromatin suggests that both the transcription and the replication of daughter DNA strands were enhanced.

In summary, the results of this study point to chromatin activation as early as 3 h after PHE. The alterations in the spectrum of histone proteins during this period of reparative regeneration set in motion a genetic program directed at enhancement of protein synthesis, renewal of cell structures, and restoration of the liver mass.

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