Volume 238, number 1, 205–210 FEB 06357 September 1988

The maximum of the histone acetyltransferase activity precedes DNA-synthesis in regenerating rat liver

Günter Weiss and Bernd Puschendorf

Institut für Medizinische Chemie und Biochemie der Universität Innsbruck, Fritz-Pregl-Strasse 3, 6020 Innsbruck, Austria

Received 11 July 1988; revised version received 12 August 1988

A pronounced transitory increase of histone acetyltransferase (EC 2.3.1.48) activity before the onset of DNA replication is shown to occur in regenerating rat liver. This effect is followed by an increase in the H1/H1° ratio at the start of DNA synthesis; H1° is replaced by H1. Histone acetylation and H1/H1° exchange are discussed as steps in the signal transduction for DNA replication.

Histone; Acetylation; DNA replication; H1° histone; Regenerating rat liver

1. INTRODUCTION

The exact biological role of histone acetylation is still unclear. This post-translational modification has been shown to affect the structure and solubility of nucleosomes [1–3]. Numerous reports describe correlations between histone acetylation and transcription [4]. Evidence for the role of histone acetylation in DNA replication and differentiation has also been presented [5,6]. Most likely histone acetylation is involved in a number of processes, perhaps all those which require a histone displacement or weakening of the histone–DNA interaction [6]. In rats after partial hepatectomy during liver regeneration a dramatic change in transcriptional activity was observed, followed by a period of DNA replication.

Since the studies by Pogo et al. [7] histone acetylation in regenerating rat liver has mainly been correlated to RNA synthesis. Little attention has been paid so far to relations between histone acetylation and DNA replication. Studies in other cell systems indicate a type of histone acetylation of pre-existing histones which precedes DNA synthesis and is discussed as a signal for the replication mechanism which is followed by acetylation of newly synthesized histones [6]. These studies were initiated in order to investigate whether similar types of replication associated activities in histone acetyltransferase can be detected in regenerating rat liver.

Correspondence address: B. Puschendorf, Institut für Medizinische Chemie und Biochemie, Fritz-Pregl-Str. 3, 6020 Innsbruck, Austria

2. MATERIALS AND METHODS

Male Sprague Dawley rats (250–320 g) were partially hepatectomized [8] or sham operated. The rats were killed at various times after hepatectomy, the livers were isolated immediately and put into 0.14 M NaCl; homogenized in 3 vols STKM (0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.9) using a Potter homogenizer, and cells were sedimented by centrifugation at 1000 × *g*. Cells were suspended in STKM containing 0.1% NP40, dounced several times and nuclei were pelleted at 1000 × *g*. Nuclei were washed two times using STC (0.34 M sucrose, 2 mM $CaCl_2$, 100 mM Tris, pH 7.9) and suspended in the same buffer at a concentration of 20 mg protein/ml. All buffers contained 10 mM Na-butyrate, 0.1 mM phenylmethylsulfonyl fluoride, 0.1% β-mercaptoethanol. All preparations of liver cells and nuclei were done on ice.

2.1. *Acetylation of histones in isolated nuclei*

Nuclei (1.2 mg protein) were preincubated in STC with 100 μM acetylcoenzyme A at 22°C for 5 min (total volume: 200 μl); the reaction was started by addition of [^{3}H]acetylcoenzyme A (1.6 Ci/mmol, final concentration 3 μM). At various

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

times (0, 1, 2, 4, 8 min) at 22°C the reaction was stopped by addition of 5 vols ice-cold STC containing 200 μM unlabelled acetylcoenzyme A. Histones were extracted with 0.2 M H_2SO_4 and precipitated by 40% trichloroacetic acid and analyzed on 8 M urea–0.46% Triton X-100 PAGE. Fluorography of the gels was performed as described [9].

2.2. *DNA synthesis*

Hepatectomized and sham operated rats were labelled by intraperitoneal injection of 1 μCi/g [^{3}H]thymidine (25 Ci/mmol). Nuclei were isolated as described above and washed with 5% trichloroacetic acid and 96% ethanol. The pellet was dissolved in 0.25 M KOH and the DNA concentration was determined according to Keck [10]. Radioactivity was measured in a liquid scintillation counter, and the specific activity of DNA was calculated.

2.3. *Incorporation of [^{3}H]acetic acid into histones*

Rats were injected intraperitoneally with 1 mCi [^{3}H]acetic acid (4 Ci/mmol) per 100 g rat 30 min before killing. Nuclei were prepared from liver as described above and histones were extracted with 0.2 M H_2SO_4, precipitated by 40% trichloroacetic acid on ice for 1 h, washed with 95% ethanol and dissolved in water. The histone concentration was determined as described by Lowry [11] and radioactivity was measured in a liquid scintillation counter.

2.4. *Preparation of H1 histones*

H1 histones were extracted from nuclei with 5% $HClO_4$ for 1 h and precipitated by acidified acetone at -20°C overnight; after centrifugation at 20000 × g for 30 min the sediments were washed with ice-cold acetone. H1 histones were dissolved in SDS sample buffer and separated on SDS gels according to Weintraub [12].

3. RESULTS AND DISCUSSION

Fig.1A shows a first maximum of the histone acetyltransferase activity in isolated nuclei 2–3 h before the start of DNA synthesis. 16–18 h after hepatectomy at the beginning of the DNA replication the enzyme activity decreases to control values of sham operated rats. The increase of specific histone acetyltransferase activity 20 h after hepatectomy (at the time of maximal thymidine incorporation) disappears, if the enzyme activity is calculated per mg histone instead of total protein (fig.1B). A second peak of histone acetyltransferase activity is observed 26 h after hepatectomy which is followed by a new round of DNA synthesis with the maximum 37 h after partial hepatectomy (fig.1B).

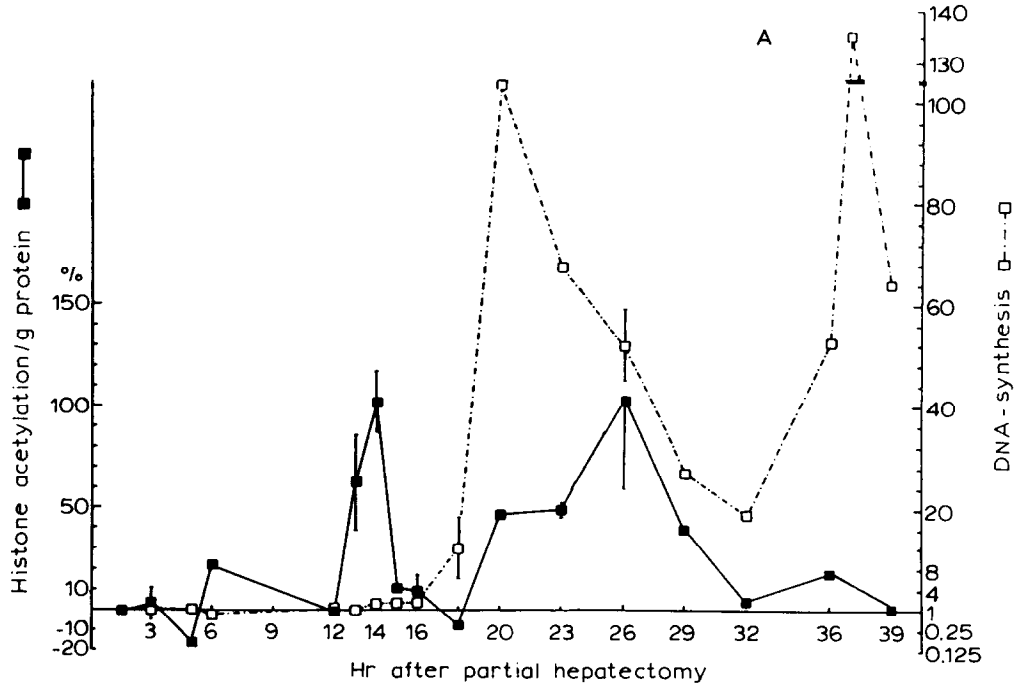
Since there is no important histone biosynthesis before the onset of DNA synthesis after hepatectomy [13] it can be concluded from our results that pre-existing histones must be acetylated before DNA synthesis can occur.

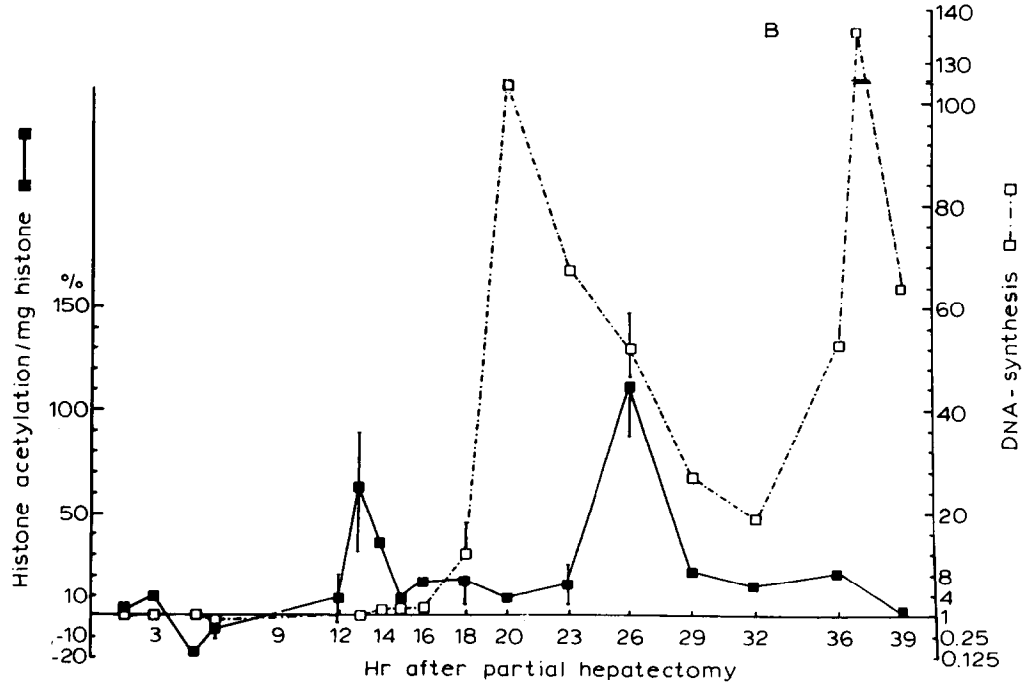
Pogo et al. [7] described an increase of [^{3}H]acetate incorporation into histones of regenerating rat liver 3–4 h after hepatectomy. We also observed a first peak of [^{3}H]acetate incorporation into regenerating rat liver 4 h after partial hepatectomy (see fig.2). After 6.5 h at the maximum of RNA synthesis [14], we detected a 60% increase of [^{3}H]acetate uptake referred to the sham operated control and nearly the same value of acetylation had been observed 12 and 18 h after partial hepatectomy, when there was still RNA synthesized. A remarkable increase of [^{3}H]acetate incorporation could be found 13 and 26 h after partial hepatectomy, at the time of the maximum of histone acetyltransferase activity. These results indicated that this increase of enzyme activity is correlated with a higher extent of acetylation at these time points. It is important to notice that there is a higher extent of [^{3}H]acetate incorporation before the onset of replication than at the maximum of RNA synthesis. The acetylation before the onset of DNA synthesis is caused by an increase of histone acetyltransferase activity whereas the first maximum of [^{3}H]acetate uptake is due to a decrease of histone deacetylase activity and not caused by an acceleration in the rate of acetylation. Our results, demonstrating no changes in histone acetyltransferase activity at these early time points after hepatectomy, are in accordance

Fig.1. (A) Acetylation of histones in isolated nuclei (pmol/min per g protein). Relative alteration in percent of [^{3}H]acetyl-CoA uptake into regenerating rat liver histones related to the sham operated control. The sham operated control (= baseline) corresponds to 3.48 ± 0.36 pmol/min per g protein. DNA synthesis in vivo: ratio of synthesis in regenerating rat liver referred to the sham operated control. Ratio 1 corresponds to 8600 ± 623 cpm/mg DNA.

$$\left[\frac{\text{cpm/mg DNA regenerating liver}}{\text{cpm/mg DNA sham operated liver}}\right]$$

(B) Acetylation of histones in isolated nuclei (pmol/min per mg histone). Relative alteration in percent of [^{3}H]acetyl-CoA uptake into regenerating rat liver histones in comparison to the sham operated control. The sham operated control (= baseline) corresponds to 0.232 ± 0.030 pmol/min per mg histone. DNA synthesis in vivo: as described above.


A
Histone acetylation/g protein
%
150
100
50
10
-10
-20
3
6
9
12
14
16
20
23
26
29
32
36
39
Hr after partial hepatectomy
DNA-synthesis
140
130
100
80
60
40
20
8
4
1
0.25
0.125


B
Histone acetylation/mg histone
%
150
100
50
10
-10
-20
3
9
12
14
16
18
20
23
26
29
32
36
39
Hr after partial hepatectomy
DNA-synthesis
140
130
100
80
60
40
20
8
4
1
0.25
0.125

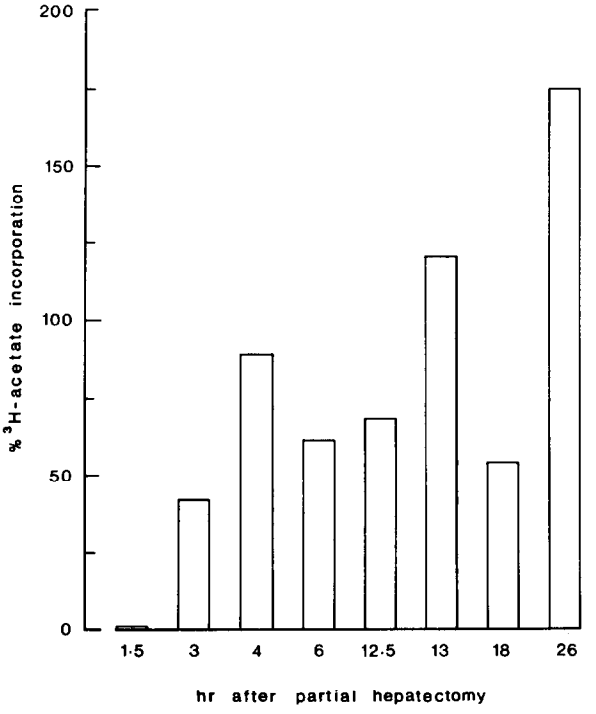


Fig.2. Relative amounts of [^{3}H]acetic acid incorporation into regenerating rat liver histones expressed as percent of the sham operated control. The sham operated control (= baseline) corresponds to 2408 ± 262 cpm/mg histone.

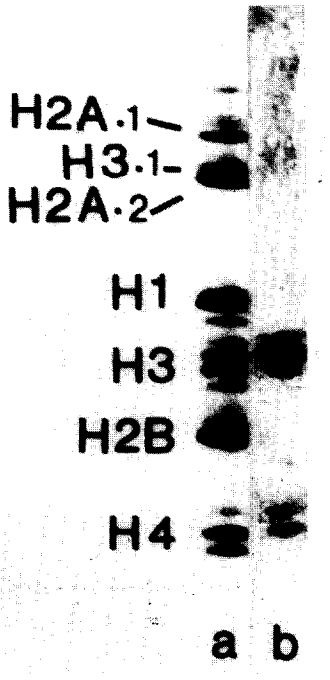


Fig.3. Electrophoretic analysis of histones isolated from rat liver nuclei 13 h after partial hepatectomy. Histones were labelled with [^{3}H]acetyl-CoA as described in section 2. (a) 8 M urea–0.46% Triton X-100 gel stained with Coomassie blue. (b) Fluorography of the same gel.

with the findings of Pogo et al. [7]. Our studies show a sharp transitory rise of histone acetyltransferase activity which occurs well beyond the first peak of RNA synthesis (6–8 h after hepatectomy) [14]. This alteration in histone acetylation which appears to be more closely related to DNA replication seems to have been overlooked so far. Similar results have been obtained with *Physarum polycephalum* in which acetylation of pre-existing histones precedes DNA replication [6].

The fluorographs (fig.3) indicate that the acetylation of H3 and H4 histones precedes that of other core histones and is observed 13–14 h after hepatectomy. Furthermore, in accordance with reports by Schröter et al. [15] these histones show the highest extent of acetylation. With regard to these reports it is suggested that the acetylation of H3 and H4 histones is preferentially correlated with the DNA replication.

After the increase of histone acetyltransferase activity there is a replacement of H1° by H1 at the start of DNA-synthesis (fig.4). It is generally assumed that the decrease of H1° is a prerequisite for the proliferation [16–18]. This assumption is supported by our data indicating a constant increase in the H1/H1° ratio during liver regeneration. This H1/H1° exchange lends additional support to the replacement hypothesis [6] which is discussed as the major function of histone acetylation. Furthermore, this H1/H1° exchange is in accordance with an involvement of histone acetylation in the alteration of the higher order structure of chromatin by an influence on the DNA linker region [19,20].

In summary, the following time-dependent sequence of events after hepatectomy is observed before the start of DNA synthesis: (1) activation of c-myc [21], (2) increase of histone acetyltransferase activity, (3) exchange of H1° by H1 by lowering the H1° content, (4) increase of DNA synthesis. All these events, which exist only for a short period,

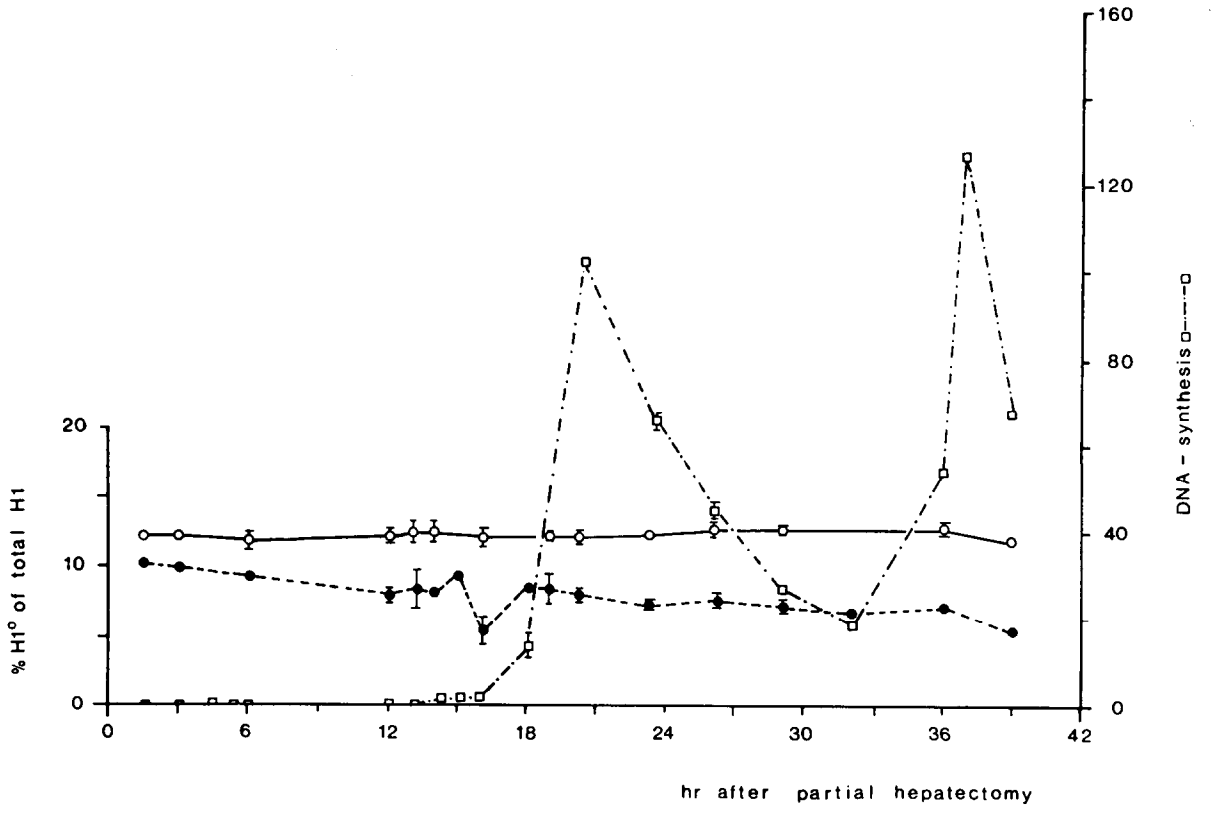


Fig.4. H1° content in percent of total H1 in regenerating (●---●) and sham operated (○—○) rat liver in relationship to DNA synthesis (see fig.1).

seem to be involved in the transduction of signals for cell proliferation after hepatectomy leading to the start of DNA replication. Further experiments in cell cultures with specific inhibitors of c-myc and/or DNA synthesis will be required in order to prove the biological relevance of the observed sequence of biochemical reactions before the start of DNA replication.

Acknowledgements: We are indebted to Professor H. Grunicke for helpful discussions and to Mrs G. Angele for preparation of the manuscript.

REFERENCES

[1] Perry, M. and Chalkley, R. (1982) J. Biol. Chem. 257, 7336–7347.
[2] Bode, J., Gomez-Liva, M.M. and Schroeter, H. (1982) Eur. J. Biochem. 130, 437–445.
[3] Ausio, J. and Van Holde, K.E. (1986) Biochemistry 25, 1421–1428.
[4] Belikoff, E., Wong, L.J. and Alberts, B.M. (1980) J. Biol. Chem. 255, 11448–11453.
[5] Doenecke, D. and Gallwitz, D. (1982) Mol. Cell. Biochem. 44, 113–128.
[6] Loidl, P. (1988) FEBS Lett. 227, 91–95.
[7] Pogo, B.G.T., Pogo, A.D., Allfrey, U.G. and Mirsky, A.E. (1968) Proc. Natl. Acad. Sci. USA 59, 1337–1344.
[8] Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186–202.
[9] Bonner, M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83–88.
[10] Keck, K. (1956) Arch. Biochem. Biophys. 63, 446–451.
[11] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[12] Weintraub, H., Palter, K. and Van Lente, F. (1975) Cell 6, 85–110.
[13] Kuehl, Le Roy (1979) J. Biol. Chem. 254, 7276–7281.
[14] Tsukada, K. and Lieberman, J. (1965) J. Biol. Chem. 240, 1731–1736.
[15] Schröter, H., Gomez-Lira, M., Plank, K.-H. and Bode, J. (1981) Eur. J. Biochem. 120, 21–28.

[16] Varrichio, F. (1977) Arch. Biochem. Biophys. 179, 715–717.
[17] P.Ca, R.A. and Valkonen, K.H. (1979) FEBS Lett. 108, 326–330.
[18] Gjerset, R., Gorka, C., Hasthorpe, S., Lawrence, J.J. and Eisen, H. (1982) Proc. Natl. Acad. Sci. USA 79, 2333–2337.
[19] McGhee, J.D., Nickol, J.M., Felsenfeld, G. and Rau, D.C. (1983) Nucleic Acids Res. 11, 4065–4075.
[20] Libertini, L.J., Ausio, J., Van Holde, K.E. and Small, E.W. (1988) Biophys. J. 53, 477–487.
[21] Makino, R., Hayashi, K. and Sugimura, T. (1984) Nature 310, 697–698.