Volume 250, number 2, 297-300 FEB 07273 July 1989

Core histone acetylation during lymphocyte activation

Aldo Profumo, Flavia Querzola and Giorgio Vidali

Istituto Nazionale per la Ricerca sul Cancro, viale Benedetto XV, 10, 16132 Genova, Italy

Received 4 April 1989

Histone acetylation has been followed in cultures of human lymphocytes, in PHA-stimulated lymphocytes and in mixed lymphocytes obtained from identical twins and from unrelated donors. A computer assisted analysis of two-dimensional gels and autoradiograms revealed that in cultured lymphocytes only H3 and H4 core histones incorporate labeled acetate and that two H3 variants greatly differ in their rate of acetate uptake.

Histone acetylation; Lymphocyte

1. INTRODUCTION

The modification of epsilon amino groups of lysine residues by acetylation is a post-synthetic reaction which takes place on all four core histone families and has attracted the attention of several investigators. Various roles have been proposed for this macroscopic event and among these histone acetylation has been related to histone deposition on newly made DNA [1,2] to gene activation [1-4] and suggested as an essential requirement for inducing a transcriptionally competent chromatin structure [5-7]. The present paper deals with a detailed analysis of histones which are the target of post-synthetic acetylation. Surprisingly, in resting or stimulated human lymphocytes only H3 and H4 incorporate labeled acetate and the results will be discussed later.

2. MATERIALS AND METHODS

Lymphocytes were purified from blood of healthy donors following established procedures [7] and the final pellet was suspended in RPMI containing 10% fetal calf serum, penicillin (50 IU/ml), streptomycin (50 μg/ml), glutamine (2 mM) at a concentration of 8×10^5 cells/ml. Stimulation of lymphocytes was obtained by adding PHA at a final concentration of 100 μg/ml or by mixing equal volumes of cell suspension obtained from different donors and in all of the following experiments 5 ml of cultured lymphocytes were used. Histones were extracted from whole cells [8] with 0.5 M HCl containing 1% 2-mercaptoethanol, 1 mM phenylmethanesulfonyl fluoride precipitated with acetone washed with ether and then air-dried. Samples were dissolved in sample buffer (8 M urea, 0.3% Triton X-100, 5% 2-mercaptoethanol, 0.9 N acetic acid) and run in the first dimension on a Triton-urea slab gel (0.3% Triton X-100, 8 M urea, 15% acrylamide, 0.9 N acetic acid). After staining in Coomassie brilliant blue, strips were cut and histones separated in a second dimension SDS-slab gel (0.1% SDS, 12.5% acrylamide). The two-dimensional gels were stained with Coomassie brilliant blue and analyzed by computer assisted densitometry in an Ultrascan XL laser densitometer LKB gel scanner. RNA synthesis in control and stimulated lymphocytes was measured by adding 0.5 μCi of [6-^{3}H]uridine (spec. act. 20-30 Ci/mmol) to 300 μl of cell suspension (in quadruplicate); and counting for the radioactivity incorporated in the TCA-insoluble material. Incorporation of labeled acetate into histones was carried out by incubating the lymphocytes for 45 min in the presence of 250 μCi of [^{3}H]acetic acid sodium salt (spec. act. 3.4 Ci/mmol). Cells were collected, histones extracted and separated by two-dimensional gel electrophoresis as described above. After staining and densitometric analysis, gels were treated with Amplify (Amersham), dried and exposed to Hyperfilm-MP (Amersham). After development, films were analyzed by computer assisted densitometry and the relative specific activities were normalized and expressed as ratios between the absorbance of the spot on the film and the absorbance of the same spot in stained gels.

Correspondence address: A. Profumo, Istituto Nazionale per la Ricerca sul Cancro, viale Benedetto XV, 10, 16132 Genova, Italy

3. RESULTS AND DISCUSSION

A sharp increase of RNA synthesis is observed

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

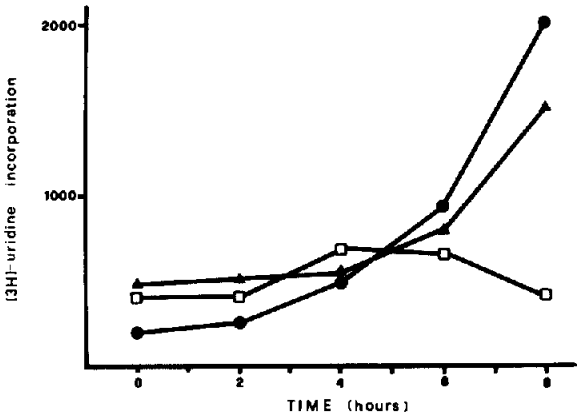

Fig.1. Rate of RNA synthesis in human lymphocytes as measured by labeled uridine uptake into TCA-insoluble material. PHA-stimulated lymphocytes (—▲—); mixed lymphocytes from identical twins (—□—); mixed lymphocytes from unrelated donors (—●—).

8 h after PHA is added to cultured purified human lymphocytes (fig.1) in agreement with experiments reported by other investigators [9]. At this time very little if any histone synthesis occurs while they are actively synthesized at later times when DNA synthesis has begun [9,10]. Given previous observations showing that increase in histone acetylation is an event preceding an augmented transcription [9,11], we have chosen to pulse the cultured cells for 60 min with labeled acetate, 4 h after the beginning of the stimulus and we have compared histone acetylation in control and stimulated cells. An example of our technical approach is reported in fig.2. In the past all four core histones were reported to be the target of the acetylating enzymes although H2A and H2B histones were found to be acetylated to a lesser extent [1,12]. In our hands the levels of labeled acetate incorporation into H2A

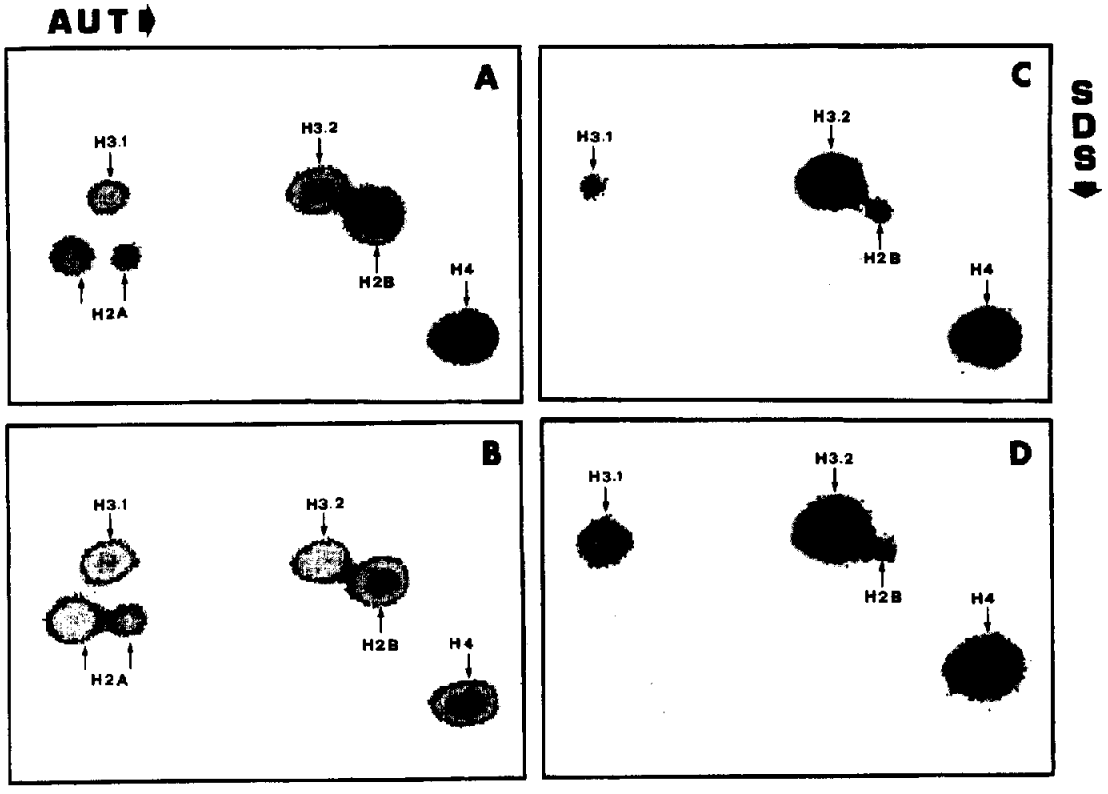

Fig.2. Computer assisted densitometric analysis of stained gels (left panels) and relative autoradiogram after pulse-labeling with [^{3}H]acetate (right panels). Control lymphocytes (panels A and C), lymphocytes after 4 h stimulation with PHA (panels B and D). The position in the gel of various histones is indicated in the figure.

Table 1

Increase in histone acetylation following PHA stimulation as calculated from the sensitometric analysis reported in fig.2

Histone	Control			PHA-stimulated			Ratio over control
	Staining	Auto-radiography	Specific activity	Staining	Auto-radiography	Specific activity	
H3.1	1.08	0.21	0.19	1.90	1.74	0.92	4.84
H3.2	2.42	3.82	1.58	2.07	4.47	2.16	1.37
H4	4.33	3.20	0.74	3.57	3.57	1.00	1.35

and H2B obtained from control as well as from PHA-stimulated lymphocytes are below the limit of detection (fig.2; panels C and D). Conversely acetylation of H3.1 histone variant which is barely detectable in control cells (fig.2, panel C) is greatly increased upon PHA stimulation (fig.2, panel D). It is also evident from fig.2 that H2A and H2B are not selectively lost during histone extraction from control (panel A) and from stimulated (panel B) cells. The experimental results which are graphically collected in fig.2 were utilized to quantitate the extent of acetate uptake in terms of specific activity and these data are reported in table 1. It is clear that stimulation with PHA increases the specific activities of H4, H3.1 and H3.2, while no radioactivity is detectable in the position where H2A and H2B migrate in our two-dimensional gel system. Not all histones, target of the acetylation, respond in the same way (table 1) and in particular the H3.1 histone variant shows a much higher response to the drug as compared to H4 and H3.2, the specific activities of the latter histones being always higher than that determined for H3.1 histone. Similar results are obtained where stimulation is induced by mixing lymphocytes obtained from unrelated donors. Table 2 summarizes these data showing a higher rate of acetate uptake in H3.1 histone as compared to H3.2 and H4. The differential rate of acetate uptake in the two H3 histone variants raised the suggestion that the two proteins showing only minor sequence diversities, may perform a different role in chromatin reorganization during lymphocyte activation. It should be pointed out that there are no amino acid substitutions in the amino-terminal regions of the two variants and therefore the substrate for histone acetylation and deacetylation enzymes is identical. It is possible however that there are changes in the environment of the actual substrate when and if long range interactions are modified by a very limited diversity in the amino sequences between the two H3 variants. No stimulation takes place in mixed lymphocyte cultures of identical twins (after a pulse with labeled acetate) and the specific activities of histones are identical, within the experimental error, to those of control lymphocytes (table 2). These results are in agreement with those reported by other investigators [12] and further confirm the possibility of using histone acetylation to evaluate the histocompatibility in organ transplantation.

Table 2

Increase in histone acetylation following stimulation in mixed lymphocyte cultures from unrelated donors

Histone	Control	Unrelated donors		Identical twins	
	Specific activity	Specific activity	Ratio over control	Specific activity	Ratio over control
H3.1	0.35	0.98	2.8	0.37	1.06
H3.2	1.85	2.18	1.18	2.01	1.09
H4	0.92	1.18	1.28	0.90	0.98

No increase in histone acetylation is observed in mixed cultures of lymphocytes obtained from identical twins

Acknowledgements: This work has been supported by the Consiglio Nazionale delle Ricerche (88.00920.44) and the Ministero della Sanita'.

REFERENCES

[1] Matthews, H.R. and Waterborg, J.H. (1985) in: The Enzymology of Post-translational Modification of Proteins, vol. 2, Academic Press, London.
[2] Jackson, V., Shires, A., Tanphaichir, N. and Chalkley, R. (1976) J. Mol. Biol. 104, 471–483.
[3] Vavra, K.J., Allis, C.D. and Gorovsky, M.A. (1982) J. Biol. Chem. 257, 2591–2598.
[4] Waterborg, J.H. and Matthews, H.R. (1983) Biochemistry 22, 1489–1496.
[5] Davie, J.R. and Candido, P.M. (1978) Proc. Natl. Acad. Sci. USA 75, 3574–3577.
[6] Vidali, G., Boffa, L.C., Bradburry, E.M. and Allfrey, V.G. (1978) Proc. Natl. Acad. Sci. USA 75, 2239–2243.
[7] Pfeffer, U., Ferrari, N., Tosetti, F. and Vidali, G. (1988) Exp. Cell Res. 178, 25–30.
[8] Wu, R.S., Tsai, S. and Bonner, W.M. (1983) Biochemistry 22, 3868–3873.
[9] Mukherjee, A.B. and Cohen, M.M. (1968) Exp. Cell Res. 54, 257–260.
[10] Cooper, H.L. (1973) in: Drugs and the Cell Cycle (Zimmerman, A.M. et al. eds) pp. 137–194, Academic Press, New York.
[11] Pogo, B.G.T., Allfrey, V.G. and Mirsky, A.E. (1966) Proc. Natl. Acad. Sci. USA 55, 805–812.
[12] Covault, J. and Chalkley, R. (1980) J. Biol. Chem. 255, 9110–9116.
[13] Mukherjee, A.B. and Fabricant, J.D. (1978) Nature 273, 467–469.