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Changes in the proportions of histone H1° subtypes in brain cortical neurons

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Histone H1° is found in tissues with little or no cellular proliferation and has been shown to accumulate during cellular terminal differentiation. Two subtypes of H1°, H1°a and H1°b, are present in any tissue where the protein has been detected. We report here the first evidence of an age-dependent change in the proportions of H1° subtypes. In rat cerebral cortex neurons the proportion of H1°a rises from 44% of total H1° at birth to about 80% at day 300. These results show that terminally differentiated neurons synthesize and exchange H1° at a significant rate.

Histone H1°; Histone H1° subtype; Histone subtype proportion; Aging; (Brain cortical neuron)

1. INTRODUCTION

Histone H1 shows a considerable degree of heterogeneity in most eukaryotic systems. In mammals, five H1 subtypes, designated H1a–e, are present [1]. It has been shown that their relative proportions change during tissue maturation [2,3]. In addition to H1a–e, all mammalian species so far examined, with the possible exception of the rabbit, contain H1° [4], first described by Panyim and Chalkley [5,6] as an H1-like protein found mainly in tissues with little or no cellular proliferation. H1° accumulation has been related to terminal differentiation in several rodent tissues [7,8]. In every tissue in which H1° has been detected, two components are present [4,9]. The two forms of H1° are very likely coded by different genes. Phosphorylation and ADP-ribosylation have been excluded as possible post-synthetic modifications responsible for the different forms [10]. Furthermore, quantitative differences have been detected in minor components of their tryptic maps [9]. The ratio of H1° subtypes has been examined in a number of tissues of several mammalian species. In rodent tissues the relative proportions of H1° subtypes differ from tissue to tissue, but appear to be similar in homologous tissues from different species [4]. The question arises as to whether the reported ratios of H1° subtypes in different tissues are affected by growth and differentiation.

We have previously shown that H1° accumulates in rat brain cortical neurons between postnatal days 8 and 18, coinciding with neuronal terminal differentiation [8]. During this period the amount of H1° in neuronal chromatin increases by a factor of about 7. The level of H1° stays constant thereafter at least up to day 300 (the last point examined). Here we report the changes in relative proportions of H1° subtypes in rat brain cortical neurons during postnatal development. This study provides the first evidence of an age-related change in the ratio of H1° subtypes.

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2. MATERIALS AND METHODS

2.1. *Isolation of neuronal nuclei and protein extraction*

Cerebral cortices from Sprague-Dawley rats were homogenized by 30 up-and-down strokes of a hand-driven Dounce homogenizer in 1 M sucrose, 1 mM sodium cacodylate, 5 mM $MgCl_2$, 1 mM dithiothreitol, 1% thiodiglycol and 10 mM sodium butyrate, pH 6.5. Cortex nuclei were fractionated according to Thompson [11]. All operations were performed at 2°C and PMSF (0.1 mM) was used throughout to inhibit proteolytic activity. The degree of contamination of the neuronal fraction with glial nuclei was about 10%, as judged by phase-contrast microscopy. Neuronal nuclei were extracted three times with 0.74 N perchloric acid as described [8].

2.2. *Polyacrylamide gel electrophoresis*

The perchloric acid extracts from neuronal nuclei were analyzed by urea-acetic acid-polyacrylamide gel electrophoresis, essentially as described by Harris and Smith [4]. Long slab gels (30 cm), containing 20% acrylamide, 0.12% bisacrylamide, 2.5 M urea and 0.9 N acetic acid were allowed to polymerise overnight and prerun for 24 h at 400 V, using 0.9 N acetic acid as running buffer. After pre-electrophoresis, the running buffer was replaced by fresh 0.9 N acetic acid and samples loaded in 8 M urea, 5% thiodiglycol, 0.1 mM dithiothreitol, 0.01% pyronin Y and 0.9 N acetic acid. Electrophoresis was run at 320 V for 26–30 h, using methyl green as tracing dye. Gels were fixed and stained with 0.25% Coomassie brilliant blue R-250 as described [8] and band intensities quantified by gel scanning at 540 nm using a Beckman DU-8B spectrophotometer.

3. RESULTS AND DISCUSSION

Histone H1° from rat brain cortical neurons can be resolved into two components, H1°a and H1°b, in long urea-acetic acid-polyacrylamide gels. These components have the same electrophoretic mobility as the corresponding bands from rat liver and glial cells (not shown).

The electrophoretic analysis of perchloric acid extracts of neuronal nuclei from rats of ages between −3 (gestational day 19) and 150 days shows

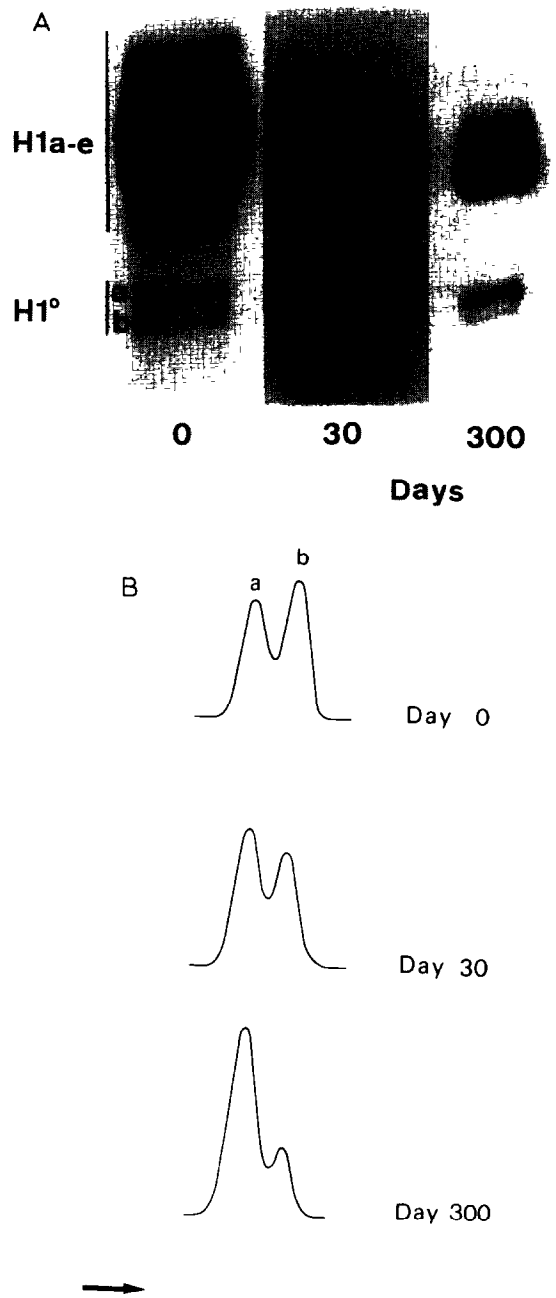

Fig.1. (A) Urea-acetic acid-polyacrylamide gel electrophoresis of perchloric acid extracts of cerebral cortex neuronal nuclei from rats of 0, 30 and 300 days. H1° subtypes a and b are indicated. The amount of protein loaded in each well was adjusted to contain an equivalent amount of H1° in all tracks. (B) Densitometric profiles of the same gel stained with Coomassie blue and recorded at 540 nm. The arrow indicates the direction of migration.

that the ratio H1°a/H1°b changes postnatally (figs 1,2). At birth the two subtypes of H1° are present in similar amounts, with a small excess of H1°b, that represents 56% of the total H1°. During development H1°b is gradually replaced by H1°a. By day 300 H1°a is clearly predominant with about 85% of total H1°. Examination of the time courses of H1°a accumulation (fig.2) suggests that the replacement of H1°b by H1°a could continue beyond day 300 and lead to the virtual elimination of H1°b in neuronal chromatin.

By contrast to H1° accumulation that appears to be restricted to a short period of approx. 10 days which coincides with terminal differentiation [8], the changes in H1° subtype proportions continue over an extended period of time. This result implies that terminally differentiated neurons synthesize and exchange H1° at a significant rate.

Our results show that the relative proportion of the subtypes H1°a and H1°b changes postnatally in rat brain cortical neurons. Similar replacement processes could also occur in other tissues such as liver and kidney. In this case, the different ratios of H1° subtypes that have been reported for several rodent tissues could result not only from

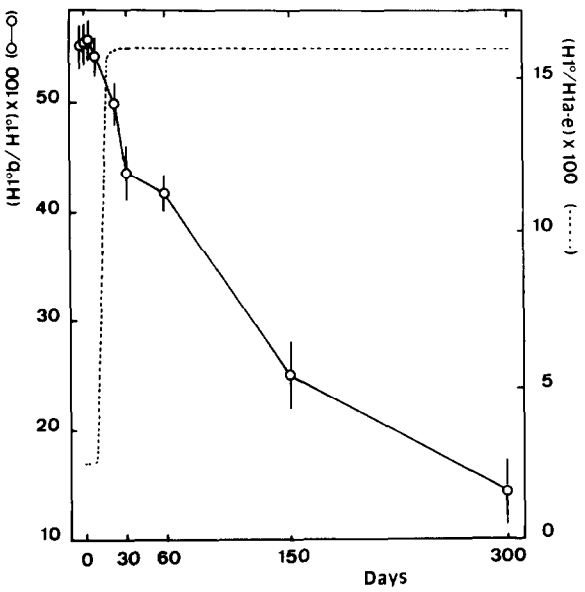

Fig.2. Time course of substitution of H1°b by H1°a, expressed as percentage of H1°b to total H1° (o—o). Values are the mean of three independent determinations. The data on H1° accumulation (·····) have been adapted from [8].

the differential expression and/or metabolic stability of the subtypes in each tissue, but also from the different stage of maturation reached in every case.

The change in histone subtype proportions during development seems to be a common feature of most eukaryotic systems. Changes in core histone variants and H1a–e subtypes during development and cell differentiation have been extensively reported in several systems [2,12]. In the case of the core histone variants the change of their relative proportions seems to be regulated at the level of synthesis [13], and most of the observed changes can be explained by different synthesis patterns in dividing and nondividing cells [12,14]. In the case of H1a–e, it has been proposed that the developmental changes in the proportions of the subtypes are mediated by both different synthesis and turnover rates in dividing and nondividing cells [2].

The proportion of H1°b does not decrease substantially during the period of H1° accumulation (fig.2) in spite of the increase of the H1° concentration by a factor of about seven, indicating that about 50% of the H1° molecules incorporated into chromatin during this period are H1°b. Therefore, although limited experimental precision precludes a direct determination of the beginning of the changes in the proportion of H1° subtypes, any change of synthesis pattern responsible for the substitution of H1°b by H1°a in neuronal chromatin should occur by the end of H1° accumulation.

The fact that the H1° subtypes undergo a process of replacement indicates that they appear as distinct for the cell at one level or another of the mechanisms involved in their synthesis or degradation. It has been suggested that H1° subtypes have separate individual functions in chromatin [4]. Our results show that the change of the proportions of H1° subtypes covers an extended period of time. Therefore, we conclude that, if the functionality of H1° is modulated by the ratio of its subtypes, the corresponding changes in chromatin function should progress gradually over a considerable fraction of the lifespan of the animal.

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REFERENCES

[1] Lennox, R.W., Oshima, R.G. and Cohen, L.H. (1982) J. Biol. Chem. 257, 5183–5189.
[2] Lennox, R.W. and Cohen, L.H. (1983) J. Biol. Chem. 258, 262–268.
[3] Lennox, R.W. (1984) J. Biol. Chem. 259, 669–672.
[4] Harris, M.R. and Smith, B.J. (1983) Biochem. J. 211, 763–766.
[5] Panyim, S. and Chalkley, R. (1969) Biochem. Biophys. Res. Commun. 37, 1042–1049.
[6] Panyim, S. and Chalkley, R. (1969) Biochemistry 8, 3972–3979.
[7] Gjerset, R., Gorka, L., Harthorpe, S., Lawrence, J.J. and Eisen, H. (1982) Proc. Natl. Acad. Sci. USA 79, 2333–2337.
[8] Piña, B., Martínez, P., Simón, L. and Suau, P. (1984) Biochem. Biophys. Res. Commun. 52, 504–510.
[9] Smith, B.J. and Johns, E.W. (1980) FEBS Lett. 110, 25–29.
[10] D'Anna, J.A., Gurley, L.R. and Becker, R.R. (1981) Biochemistry 20, 4501–4505.
[11] Thompson, R.J. (1973) J. Neurochem. 21, 19–40.
[12] Zweidler, A. (1980) in: Histone Genes (Stein, G.S. et al. eds) pp.339–371, Wiley, New York.
[13] Schümperli, D. (1986) Cell 45, 471–472.
[14] Wu, R.S., Tsai, S. and Bonner, W.M. (1982) Cell 31, 367–374.