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Histone H1 subtype synthesis in neurons and neuroblasts

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Summary

Rat cerebral cortex neurons contain the five histone H1 subtypes H1a-e and the subtype H1° present in other mammalian somatic tissues. The four subtypes H1a-d decay exponentially during postnatal development and are partially or totally replaced by H1e that becomes the major H1 subtype in adults. H1° accumulates in a period restricted to neuronal terminal differentiation. Here we study the synthesis of the H1 subtypes in cortical neurons and their neuroblasts by *in vivo* labeling with [^{14}C]lysine. The subtype synthesis pattern of neuroblasts has been determined by labeling gravid rats during the period of proliferation of cortical neurons and synthesis in neurons has been studied by postnatal labeling. The subtype H1a is synthesized in neuroblasts but not in neurons and is therefore rapidly removed from neuronal chromatin. The synthesis of H1b and H1d is much lower in neurons than in neuroblasts so that these subtypes are replaced to a large extent during postnatal development. H1c is synthesized at levels much higher than the other subtypes both in neurons and neuroblasts, but its very high turnover, about one order of magnitude faster than that of H1e in neurons, favors its partial replacement during postnatal development. Comparison of the synthesis rates of H1° in newborn and 30-day-old rats shows that the accumulation of H1° in differentiating neurons is due to an increased level of synthesis.

Key words: histone H1, subtype, differentiation, neuron, neuroblast.

Introduction

Histone H1 is necessary for the induction and stability of chromatin higher order structure. The H1 histone class is coded by a multigene family. The somatic H1 fraction from mammals has been resolved by two-dimensional electrophoresis in five subtypes, H1a, b, c, d and e (Cole, 1984; Lennox et al., 1982; Lennox and Cohen, 1983; Lennox, 1984). In addition to these subtypes, some somatic tissues contain H1° (Panyim and Chalkley, 1969). The synthesis of H1° is greater in tissues with little or no cell division (Gerset et al., 1982). The H1 subtypes differ in extent of phosphorylation and in evolutionary and metabolic stability (Lennox and Cohen, 1983; Lennox, 1984). The subtypes also differ in their relative rates of synthesis and degradation in proliferating and quiescent cells (Lennox and Cohen, 1983; Pherson and Cole, 1982). In addition, it has been shown that some of the subtypes differ in their ability to condense DNA *in vitro* (Welsh and Cole, 1979, 1980; Liao and Cole, 1981). These properties have led to the suggestion that not all subtypes are equally efficient in promoting chromatin higher order structures (Lennox, 1984; Huang and Cole, 1984).

In previous papers, we showed that in brain cortical neurons the proportions of the H1 subtypes change during postnatal development. The subtypes H1a-d decay exponentially and are replaced to different extents by H1e, which becomes the most abundant subtype in mature neurons. The changes affecting the composition in the subtypes H1a-e are already apparent at birth and the subtype composition is close to steady-state by postnatal day 30 (Piña et al., 1987). H1° is differently regulated. It accumulates in a restricted period between days 8 and 18, coinciding with neuronal terminal differentiation, and its concentration remains stable thereafter (Piña et al., 1984). The replacement of the subtypes H1a-d by H1e during postnatal development suggests that neurons and neuroblasts have distinct synthesis patterns. The purpose of this study has been to compare the H1 subtype synthesis patterns of neurons and neuroblasts in order to explain the changes in subtype composition in neurons during postnatal development.

Mammalian cortical neurons do not divide after birth (Jacobson, 1978). The incorporation of newly synthesized histones into neuronal chromatin can thus be followed by means of postnatal injections of labeled amino acid precursors. The study of histone synthesis in neuroblasts is not so straightforward because these germinal cells cannot be isolated in enough quantities for analysis. We have devised a labeling format that

allows access to histone synthesis in neuroblasts. For this purpose gravid rats were injected with [^{14}C]lysine during the period of proliferation of the brain cortical neurons of the fetuses and the labeled histone incorporated in the dividing neuroblasts recovered from the neurons of the newborn. The method is reminiscent of that of Angevine and Sidman (1961) that permitted the establishment of the time of origin of neurons in the mammalian cerebral cortex. In this procedure, the gravid female was injected with tritiated thymidine, and the pattern of labeled neurons in the fetuses was determined postnatally at various intervals after injection. The relatively long half-life of DNA-bound H1 together with the very well-defined timing of proliferation of cortical neurons have made it possible to adapt this procedure to the study of histone synthesis in neuroblasts.

Materials and methods

In vivo *labeling procedures*

In order to study the synthesis of the H1 subtypes in neuroblasts, gravid Sprague-Dawley rats were injected intraperitoneally once a day with 0.28 μCi of L-[^{14}C]lysine per gram of body weight on days 16, 17, 18, 19, 20 and 21 of gestation The rats were born on day 22 and the protocol for histone isolation and histone extraction began two hours after birth.

For the study of the synthesis of the H1 subtypes in neurons, either newborn or 30-day-old rats were injected intraperitoneally three times with doses of 2 μCi of L-[^{14}C]lysine per gram of body weight at 4 hour intervals and killed 14 hours after the last injection.

Isolation of neuronal nuclei

The cerebral cortices were homogenized by hand with 30 up-and-down strokes of a Dounce homogenizer in 1 M sucrose, 1 mM sodium cacodylate, 5 mM $MgCl_2$, 1 mM dithiothreitol, 1% thiodiglycol, pH 6.5. Cortex nuclei were fractioned according to Thomson (1973). All operations were performed at 2°C and phenylmethylsulfonyl fluoride (0.1 mM) was used throughout to inhibit proteolytic activity. The degree of contamination of the neuronal fraction with glial nuclei was of the order of 10%, as judged by phase-contrast microscopy.

H1 extraction

The pellets of neuronal nuclei were resuspended in 10 mM EDTA, 1% thiodiglycol, pH 7.4 and sonicated. Perchloric acid-insoluble material was precipitated by addition of concentrated $HClO_4$. The combined supernatants containing H1 were acidified by addition of concentrated HCl up to 0.25 M and proteins precipitated overnight at −20°C by addition of 6 vol. of cold acetone. Proteins were collected by centrifugation. The pellet was washed with acetone/thiodiglycol/HCl (97:2:1) and acetone/thiodiglycol (98:2) and vacuum dried.

Gel electrophoresis

Two-dimensional gel electrophoresis was performed basically as previously described (Piña et al., 1987). The first dimension was a 30-cm-long urea/acetic acid gel, and the second a modified SDS gel according to Lennox and Cohen (1984), in which the separating gel contained 15% acrylamide and 0.56% bisacrylamide, and the stacking gel contained 6% acrylamide with the pH reduced to 6.5 Gels were stained for 24 hours with Coomassie brillant blue R-250 in methanol/water/acetic acid (5:5.1) and destained in methanol/ethanol/water/acetic acid (7:1:1:1). Fluorograms were prepared according to the method of Laskey and Mills (1975).

Measurement of the spot intensities

Fluorograms were measured by densitometry using a VINIX system for the digital processing of the images.

Results

Labeling formats

Neuroblast labeling

In the rat brain, cortical neurons are generated continuously by mitosis of germinal cells in a period that goes from intrauterine day 16 to shortly before birth on gestational day 22 (Berry et al., 1964). In order to establish the H1 subtype synthesis pattern in dividing neuroblasts, gravid rats were injected intraperitoneally with [^{14}C]lysine during the proliferation period of the brain cortical neurons of the fetuses, that is on days 16 through 21 of gestation. The labeled histone synthesized in dividing neuroblasts was recovered from the neuronal fraction of the newborn. Although histones are synthesized outside the S-phase of the cell cycle, most histone synthesis is tightly linked to S-phase (Wu and Bonner, 1981; D'Incalci et al., 1986). Therefore, the H1 prenatally labeled should essentially correspond to that synthesized and incorporated onto the DNA during the S-phase of dividing neuroblasts.

Neuron labeling

The synthesis pattern of neurons was studied by postnatal labeling. The absence of DNA replication in neurons makes the incorporation of newly synthesized histone molecules onto neuronal DNA rate limited by the turnover of the DNA-bound histone.

Synthesis of the H1 subtypes in neuroblasts

The synthesis pattern of dividing neuroblasts was obtained by prenatal labeling and the H1 fraction was purified from the neuronal nuclei of the newborn. The fluorography shows that all subtypes are incorporated in dividing neuroblasts (Fig. 1A). The intensities increase in the order H1°<H1a<H1b≈H1d<H1e<H1c, according to the proportions shown in the histogram of Fig. 3. The most striking feature of this pattern is the high incorporation of H1c, which is equivalent to that of all other subtypes taken together.

The subtype composition of the H1 newly incorporated onto the DNA during S-phase is basically determined by the relative synthesis rates of the subtypes. A steady-state for the H1 subtype composition of newly replicated chromatin cannot, however, be defined because as soon the chromatin assembly process is completed the representation of the different subtypes will either increase or decrease according to the balance of synthesis and turnover rates. In the case of the H1 subtypes, the balance favors the accumulation of H1e and the replacement to different extents of all the other subtypes during postnatal development.

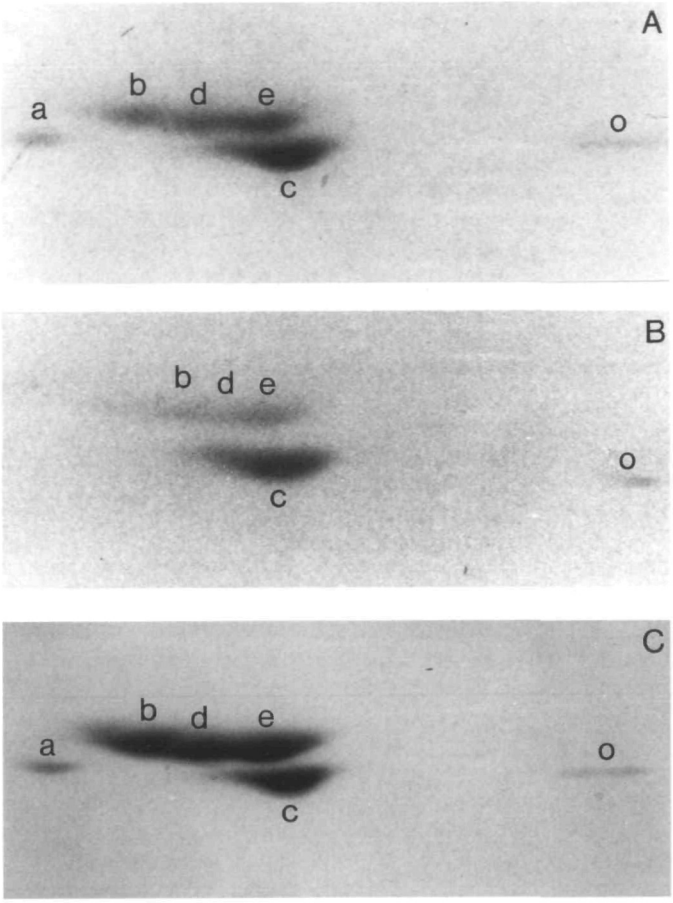

Fig. 1. Mass and synthesis patterns of the histone H1 subtypes of rat brain cortical neurons and their neuroblasts H1 subtypes were analyzed by two-dimensional electrophoresis as described in Material and methods. (A) Fluorography showing the synthesis pattern of neuroblasts obtained through prenatal labeling of a gravid rat with [^{14}C]lysine during the period of proliferation of the cortical neurons of the fetuses. (B) Synthesis pattern of neurons from newborn animals. (C) H1 fraction from newborn animals stained with Coomassie blue.

Considering that the half-life of brain H1 has an average value of 13 days (Duerre and Lee, 1974), the fluorographic pattern obtained with prenatal labeling can be regarded as a good approximation of the subtype composition of the H1 newly incorporated into chromatin. The case of H1c deserves special consideration as this subtype has a half-life shorter than the average. A value as short as 2 1/2 days can be calculated from data of Pherson and Cole (1982) on mouse neuroblastoma. With such a half-life, about 45% of the H1c incorporated during S-phase should be lost by exchange. However, these exchange losses are replaced to a large extent because H1c is also actively synthesized in neurons as described in the next section.

Synthesis of the H1 subtypes in neurons

Neuron proliferation in the rat cortex stops shortly before birth. Neuronal synthesis patterns can therefore be studied by postnatal labeling without interference from cell proliferation. The synthesis pattern obtained by postnatal labeling of newborn rats is clearly distinct from that of neuroblasts. H1a is not synthesized in neurons, the synthesis of H1b decreases to very low levels and that of H1d also decreases, although to a lesser extend. In contrast, H1c and H1e are incorporated at levels equivalent to those observed in neuroblasts. H1c dominates the pattern with about 50% of the total incorporation.

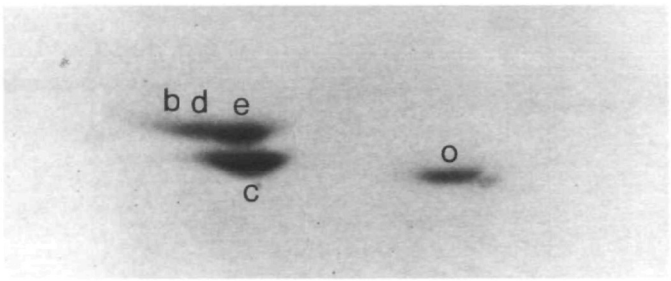

Fig. 2. Synthesis pattern of the H1 subtypes in brain cortical neurons from 30-day-old rats. Rats were labeled with [^{14}C]lysine. The fluorograph was obtained as described in Materials and methods.

Fig. 2 shows the pattern of synthesis obtained when rats were labeled at postnatal day 30, when terminal differentiation of cortical neurons is completed and the composition in subtypes, including H1°, is already close to steady-state (Piña et al., 1984, 1987). The pattern of incorporation of the subtypes H1b-e is identical within the statistical error to that of the newborn. In contrast, the level of incorporation of H1° is much higher, indicating that the accumulation of H1° in differentiating neurons is a simple consequence of a higher level of synthesis. This result also shows that H1° turns over in mature neurons.

The mass pattern of the H1 sample isolated from newborn animals was obtained with Coomassie blue staining (Fig. 1C). It shows that all H1 subtypes are present in the chromatin of the newborn. H1b, H1c, H1d and H1e are present in similar amounts, varying between 21% and 26%. H1a represents less than 5%. A very small amount of H1° is also present. This subtype composition is still far from equilibrium and thus further modified during postnatal development. Beyond postnatal day 60, when H1b, H1c, H1d and H1e represent, respectively, 6%, 16%, 9% and 69% of total H1, the subtype composition remains stable as reported by Piña et al. (1987).

The relationship between the relative levels of incorporation of the subtypes in mature chromatin (Fig. 3) and the steady-state proportions reported by Piña et al. (1987) gives an estimation of the relative turnover rates of the whole set of subtypes present in neurons. It can be seen in Table 1 that the fastest turnover corresponds to H1c, which turns over one order of magnitude faster that H1e, whereas H1b, H1d and H1° occupy intermediate positions.

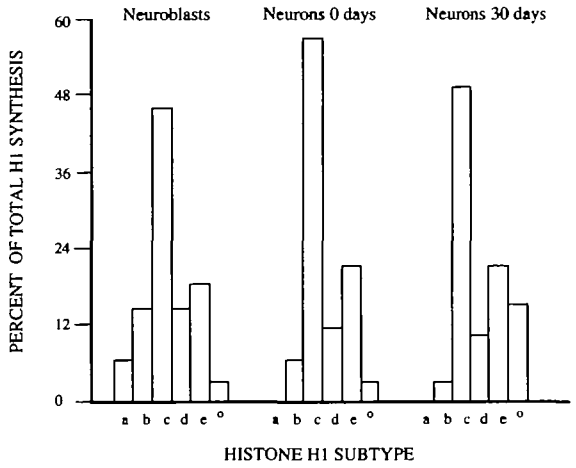

Fig. 3. Measurement of the H1 synthesis patterns of neurons and neuroblasts. The intensities were obtained by densitometry of digitalized images.

Table 1. *Relative turnover rates of histone H1 subtypes*

Subtype	% of total H1	% of total synthesis	Relative turnover
H1a	–	–	–
H1b	5	3	0 60
H1c	14	50	3.57
H1d	8	10	1 25
H1e	59	22	0 37
H1°	14	15	1.07

The percentages of total H1 in rat brain cortical neurons were taken from Piña et al. (1984) and Piña et al. (1987) and correspond to the subtype proportions at postnatal day 150. The relative turnover rates of the subtypes were calculated as the quotients of the relative levels of synthesis and the corresponding steady-state proportions

Discussion

The proportions of the H1 subtypes have been shown to change during postnatal development in brain cortical neurons (Piña et al., 1984, 1987). The changes in subtype composition can be explained by comparing the synthesis patterns of neurons and neuroblasts. H1a is synthesized in neuroblasts but not in neurons and is thus rapidly removed from neuronal chromatin. The decrease of H1b and H1d is due to a lower synthesis level in neurons. The high level of synthesis of H1c together with its low concentration in chromatin indicates that this subtype has a high turnover rate. Considering the steady-state concentrations and the relative synthesis rates of H1c and H1e in mature neurons, we can estimate that H1c turns over about one order of magnitude faster than H1e, which is the subtype with the slowest turnover. The accumulation of H1° during neuronal terminal differentiation is due to an increased level of synthesis as shown by the comparison of the synthesis patterns from inmature and terminally differentiated neurons. Differential synthesis of H1 subfractions has previously been reported by Sizemore and Cole (1981) and D'Anna et al. (1982; 1985). D'Incalci et al. (1986) also observed that in quiescent human fibroblasts the synthesis of three H1 subtypes was greatly decreased. These subtypes presumably correspond to H1a, H1b and H1d. However, a definitive assignment has not been possible because these authors used a gel system different from that of this study.

The subtype composition of the H1 newly incorporated onto the DNA in S-phase is determined by the relative concentrations of the different subtypes available for binding to DNA, which in turn should be determined by the corresponding synthesis rates. The latter are established with good approximation by neuroblast labeling. Subsequently, differential turnover also contributes to the definition of the subtype composition of chromatin together with the changes in synthesis pattern associated to neuronal commitment. It is important to emphasize that even if the synthesis patterns of neurons and neuroblasts were identical, differential turnover would cause by itself the replacement of some subtypes by others during postnatal development. The role of differential turnover in the control of H1 subtype proportions was given considerable emphasis in studies on butyrate treated neuroblastoma cells (Hall and Cole, 1985). It is apparent from the results that there is no single cause explaining the changes in H1 subtype proportions in neuronal chromatin during postnatal development. H1a is removed from neuronal chomatin as a consequence of the strict dependence of its synthesis on DNA replication. H1b and H1d are less dependent on DNA replication because, although their synthesis rates are greatly decreased in neurons, they remain present as minor components in mature chromatin. H1c and H1e are not dependent on DNA replication and their relative synthesis rates are similar in neurons and neuroblasts. However, in this case, the much higher turnover of H1c favors the accumulation of H1e and a slight decrease of H1c during postnatal development.

Several lines of evidence indicate that the H1 subtypes differ in their capacity to condense DNA *in vitro*. Results of Huang and Cole (1984) show that these differences are likely to be relevant to chromatin condensation in nuclei. On the basis of metabolic and developmental considerations, Lennox (1984) suggested that H1c would be much less efficient than the other subtypes in promoting chromatin higher order structure. If it was so, a clearer expression of the properties of H1c should be found in newly replicated chromatin before its concentration has been leveled down as a result of its high turnover. In contrast, the subtype composition of mature chromatin is dominated by H1e, the subtype with the slowest turnover, which in neuronal chromatin reaches nearly 70% of total H1.

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