CHANGING PATTERNS OF HISTONE ACETYLATION AND RNA SYNTHESIS OF THE DEVELOPING AND AGEING RAT BRAIN

H.-I. SARKANDER and E. KNOLL-KÖHLER

Department of Pharmacology of the Free University of Berlin, 1000 Berlin 33, Thielallee 69–73, Germany

Received 6 November 1977

1. Introduction

In essence most of the current theories of ageing (e.g., error theory, mutation theory, crosslinking theory, immunologic theory, radical theory) anticipate that the numerous biochemical parameters, which vary as a function of age, reflect modifications in the expression of genetic information encoded in DNA [1–3].

At the transcriptional level of gene expression a great deal of attention has been devoted to post-synthetic modifications of DNA-associated proteins as one of the main means of directed regulation for modulating the outflow of genetic information [4,5]. The implication is that post-synthetic modifications of chromatin-bound proteins induce changes in DNA conformation affecting the binding of enzymes and proteins related to specific gene expression and contributing to the structure of different chromatin forms [6,7]. Among the post-synthetic modifications the process of acetylation of chromatin-bound histones has been observed to correlate closely with gene activation for RNA synthesis [4,8]. It becomes increasingly clear, that histones serve to restrict the number of RNA initiation sites on chromatin and that structural modifications of histones by acetylation are involved in the regulation of the degree of the genetic restriction of chromatin by altering the number of RNA initiation sites accessible on chromatin for endogenous RNA polymerase molecules [9,10,22].

Since the well-defined changes in the structure and transcription of chromatin during ontogenetic development are most probably under genetic control [11–13] the question arises as to whether the changing patterns of histone acetylation occurring during the life span of a cell [14,15] are functionally implicated in the ontogenetic variations of chromatin transcription.

In this study on rats of different ages, the in vitro uptake of acetate into chromatin-bound histones and the chromatin-templated RNA synthesis of isolated brain nuclei were measured in order to assess the significance these parameters may possess in terms of the biological function of chromatin for, or concomitant with, ontogenetic development. It will be shown, that there are clear age-dependent differences in the rate of histone acetylation coinciding with altered levels of RNA synthesis.

2. Methods

2.1. *Isolation of nuclei*

Virgin Wistar albino rats strain SW 70 (Winkelmann Breeding Co. Borchen, FRG) were used for all experiments. The animals were kept under controlled conditions (defined light–dark rhythm, 10 h light, 26°C, 60% air humidity). Mating took place within a 2 h period from 7:00–9:00 a.m.. The first 24 h period after mating was called day 0 of pregnancy if sperms were detected in the vaginal smear. On day 14–20 of pregnancy the rats were killed by decapitation (9:00–10:00 a.m.) and after dissecting the embryos from the uteri the brains were rapidly removed and dissected in different regions. The preparation of nuclei from pooled regions of embryonal rat brains as well as from newborn and adult rat brains was carried out as in [16,17].

2.2. *Conditions for RNA synthesis*

Endogenous RNA polymerase activities were measured under conditions completely eliminating reinitiation using the technique in [9]. To allow formation of stable initiation complexes cerebral nuclei (about 5 μg DNA) were incubated for 15 min at 37°C in 50 μl assays containing (final concentration): 50 mM Tris–HCl, pH 7.9; 3 mM $MnCl_2$; 1.0 mM each ATP and GTP; and 0.03 mM [^{3}H]UTP (spec. act. 0.83 mCi/mmol). Then RNA chain elongation was permitted for additional 15 min by addition of 1.0 mM CTP and $MgCl_2$ and $(NH_4)_2SO_4$ in final concentrations of 5.0 mM and 400 mM, respectively. The final UMP incorporation into cerebral nuclear RNA was determined by the paper disk method as in [8].

2.3. *Acetylation of cerebral histones*

Histones were isotopically labeled by incubating cerebral nuclei isolated from rats at different stages of development with saturating amounts of [^{3}H]-acetyl-coenzyme A (spec. act. 0.50 Ci/mmol) in assay mixtures consisting of 0.32 M sucrose, 20 mM Tris–HCl, pH 7.5, 3 mM $MgCl_2$ and 6 mM $Na_2S_2O_5$ for preventing degradation of histones by proteases. After an incubation period of 40 min at 37°C when histones were acetylated up to maximum values [17] aliquots of the assay mixtures were pipetted into ice-cold H_2SO_4 up to a final concentration of 0.4 N and histones were extracted and purified as detailed in [8,17].

2.4. *Gel electrophoretic separation of acetylated histone fractions*

Histones were characterized by electrophoresis in 15% polyacrylamide gels containing 3.125 M urea following the method in [18] with the exception that ethylene diacrylate was used as a crosslinking agent instead of methylene bisacrylamide. After dissolving the cerebral rat brain histones in 0.9 N acetic acid the isotope distribution in the different histone fractions was examined by separating under identical conditions equal amounts of [^{3}H]acetylated histone samples followed by staining, destaining, scanning, solubilizing and radioanalyzing the gels as detailed in [8,21].

2.5. *Analytical procedures*

Protein was assayed by the method in [19] and DNA was determined by the diphenylamine reaction as modified [20].

3. Results and discussion

In eukaryotes chromatin is organized in a manner allowing for regulation of the expression of the genetic information encoded in DNA. Transcription of chromatin by endogenous DNA-dependent RNA polymerases (EC 2.7.7.6) undergoes sequential variations during different developmental stages of a tissue or during the life cycle of a cell [11–13,26]. Insight into the regulatory mechanisms by which such transcriptional changes may be brought about is limited by our understanding of the structural and biochemical interaction of the nuclear components. In part, ontogenetic variations of transcription may be attributable to post-synthetic modifications of DNA-associated proteins such as acetylation, phosphorylation, methylation or thiolation, altering the mode of association of modified chromosomal proteins with corresponding DNA interaction sites and other chromatin components [4,5,7,16].

Allfrey et al. were first to correlate changes in covalent modifications of chromosomal proteins by acetylation and phosphorylation to changes in nuclear RNA synthesis (reviewed [4]). We found that following the administration of growth-inducing drugs an increased acetylation of histones preceded and accompanied an increased RNA synthesis [8,21]. Conversely, the lanthanide-induced inhibition of rat liver RNA synthesis is temporally connected with an inhibition of acetate uptake by chromatin-bound liver histone fractions [10]. Lately, we reported a positive correlation between the degree of neuronal and glial histone acetylation and the degree of the number of RNA initiation sites accessible for RNA polymerase molecules on neuronal and glial rat brain chromatin [17,22]. Although direct evidence is lacking the results of these and other studies are generally consistent with the view that there is a functional relationship between histone acetylation and RNA synthesis.

In the present study on cerebral rat brain nuclei isolated at different stages of ontogenesis we focused on correlated changes between modifications of transcription and covalent modifications of histones by acetylation.

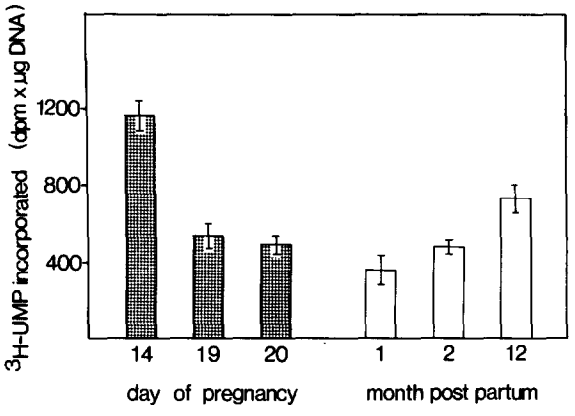

Fig.1. Incorporation of [^{3}H]UMP into nuclear RNA by cerebral rat brain nuclei isolated at different stages of pre- and post-natal development. Components of assays and incubation conditions were the same as in [9] for initiation and elongation of nuclear in vitro RNA synthesis and given in section 2 including 0.03 mM [^{3}H]UTP/assay set. Values given are means of triplicate determinations ± SEM.

A comparison of the data in fig.1 indicates that the greatest changes in nuclear cerebral in vitro RNA synthesis, i.e., UMP incorporation into cerebral nuclear RNA take place during the embryogenesis, especially between day 14 and 20 of gestation. Coincidentally the rate of [^{3}H]acetate uptake by chromatin-bound histones decreased directly proportional to the altered levels of RNA synthesis. As further summarized in fig.1, cerebral nuclear in vitro RNA synthesis did not vary significantly between day 19 and 20 of pregnancy. From the comparative gel-electrophoretic analysis of [^{3}H]acetylated histone fractions it is apparent that the decrease of acetate uptake during the fetal development mainly concerns the arginine-rich fractions H3 and H4 whereas the fractions H2B and H2A seem to be changed to a lesser extent (fig.2). On the other hand measuring of histone acetylation of chromatin-bound cerebral histones and chromatin-templated RNA synthesis in nuclei isolated from 1–12-month old rats established that both biochemical processes were higher in old rats when compared with the values obtained using the younger rats (fig.1,2).

These findings contradict reports suggesting that the acetylation rate of histones decreases with age [14]. This discrepancy could be due to employing different tissues with different mitotic rates influencing the parameters investigated as cited by [23] and/or due to age-related changes in acetate pool size and/or acetate metabolism which must be excluded when using acetate instead of the direct acetate donator acetyl-coenzyme A. Our present results fully agree with [15,24] indicating that the intensity of acetylation of liver histones is greater in old than in young rats. Cerebral nuclei from adult rats exhibited a greater

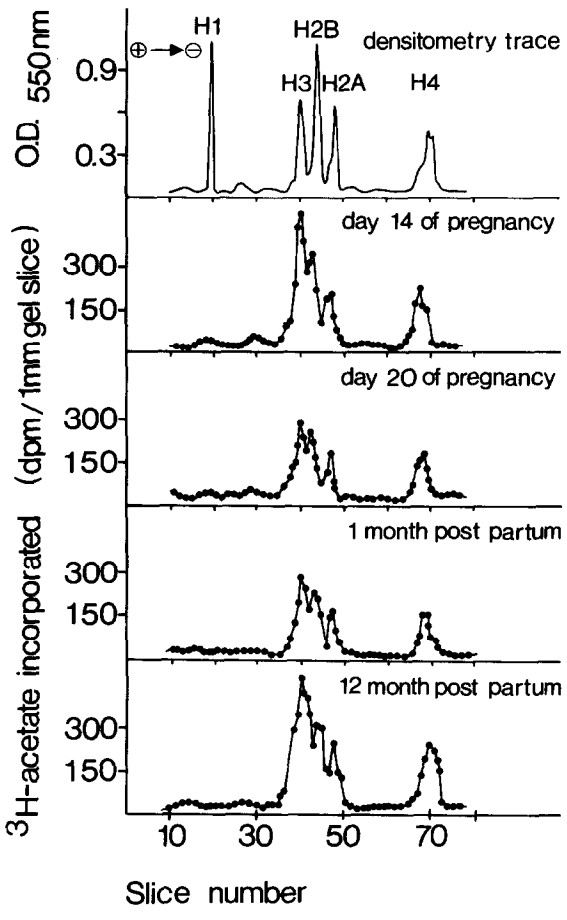

Fig.2. Altered rates of cerebral rat brain histone acetylation at different stages of development. Chromatin-bound histones were isotopically labeled up to maximum values by incubating cerebral nuclei isolated at the times indicated with 8.7 µCi [^{3}H]acetyl-coenzyme A (spec. act. 0.50 Ci/mmol) for 40 min as in [17]. Polyacrylamide gel electrophoretic separation of [^{3}H]acetylated histone fractions was carried out using the method in [18] followed by staining, destaining, scanning, slicing, solubilizing and radioanalyzing the gels as detailed in [8].

histone acetylating activity than did the corresponding preparation from newborn animals.

Since the sequence-specific acetylation of ϵ-NH_2-lysine residues of histones occur at their primary DNA binding sites the degree of acetylation is an assayable function of the number of histone binding sites not bound to the PO_4-groups of the DNA backbone. Thus, changing patterns of acetylation in the developing and ageing rat brain reflect changes in the mode and tightness of histone binding to DNA supposed to lead to modifications of structure and function of chromatin [4,6]. In particular, since studies using fractionated chromatin revealed that histones are bound differently in repressed and transcriptable chromatin fractions as indicated by a 2-fold higher histone acetylation rate in the transcriptable portion of chromatin [25] changing patterns of histone acetylation during the ontogenesis appear to be an indication of changes in the ratio of transcriptable to repressed chromatin within the genome.

In conclusion, if regulatory changes of chromatin function are responsible for the sequential changes of biochemical parameters occurring during the various phases of life span our results will be of interest in providing a structural basis for regulatory changes in the transcriptional properties of chromatin. It must, however, be emphasized that at present our study represents a temporal relationship and does not prove that age-related modification of histone acetylation is a causative factor which leads to the corresponding age-related transcriptional changes. Moreover, it remains to be unequivocally established whether or not these changes are a cause or only an accompanying event of the developmental and ageing processes. Criteria other than the chronological age are required to determine the specifity of different alterations to the process of ageing.

Acknowledgements

We acknowledge the excellent technical assistance of Mr Frank Wojnorowicz.

References

[1] Orgel, L. E. (1963) Proc. Natl. Acad. Sci. USA 49, 517–521.
[2] Comfort, A. (1974) Mech. Age. Dev. 3, 1–31.
[3] Kanungo, M. S. (1976) in: Interdiscipl. Topics Geront. (Cutler, G. C. ed) Vol. 10, pp. 100–107, Karger, Basel.
[4] Allfrey, V. G. (1971) in: Histones and Nucleohistones (Phillips, D. M. P. ed) pp. 241–294, Plenum, New York, London.
[5] Stein, G. S., Spelsberg, T. C. and Kleinsmith, L. J. (1974) Science 183, 817–824.
[6] Hnilica, L. S. (1972) in: The structure and biological functions of histones, pp. 79–92, Chemical Rubber Co. Press, Cleveland.
[7] Elgin, S. C. R. and Weintraub, H. (1975) Ann. Rev. Biochem. 44, 725–774.
[8] Sarkander, H.-I. and Brade, W. P. (1975) Biochem. Pharmacol. 24, 2279–2285.
[9] Axel, R., Cedar, H. and Felsenfeld, G. (1974) Cold Spring Harbor Symp. Quant. Biol. 38, 773–783.
[10] Sarkander, H.-I. and Brade, W. P. (1976) Arch. Toxicol. 36, 1–17.
[11] Wulff, V. J., Samis, H. V. and Falzone, R. S. (1970) Adv. Gerontol. Res. 2, 37–76.
[12] Cutler, R. G. (1975) Expl. Gerontol. 10, 37–60.
[13] Hahn, H. P. von (1970) Gerontologia 16, 116–128.
[14] Ryan, J. M. and Cristofalo, V. J. (1972) Biochem. Biophys. Res. Commun. 8, 735–742.
[15] Liew, C. C. and Gornall, A. G. (1975) Fed. Proc. Fed. Am. Soc. Exp. Biol. 34, 186–187.
[16] Sarkander, H.-I. and Uthoff, C. G. (1976) FEBS Lett. 71, 53–56.
[17] Sarkander, H.-I., Fleischer-Lambropoulos, H. and Brade, W. P. (1975) FEBS Lett. 52, 40–43.
[18] Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337–346.
[19] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1961) J. Biol. Chem. 193, 265–275.
[20] Burton, K. (1956) Biochem. J. 62, 315–323.
[21] Sarkander, H.-I., Kemmerle, M. and Brade, W. P. (1974) Naunyn-Schmiedeberg's Arch. Pharmacol. 284, 39–53.
[22] Sarkander, H.-I. and Dulce, H.-J. (1978) Exp. Brain Res. in press.
[23] Pogo, B. G. T., Allfrey, V. G. and Mirsky, E. A. (1966) Proc. Natl. Acad. Sci. USA 55, 805–812.
[24] Bondy, S. C., Roberts, S. and Morelos, B. S. (1970) Biochem. J. 119, 665–672.
[25] Simpson, R. T. (1973) Adv. Enzymol. 38, 41–108.
[26] Giuffrida, A. M., Cox, D. and Mathias, A. P. (1975) J. Neurochem. 24, 749–755.