Vol. 109, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 16, 1982 Pages 78-82

THE DISTRIBUTION OF THE HISTONE H1° IN DIFFERENT BRAIN CELL TYPES

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Received September 14, 1982

H1° levels were examined in two populations of nuclei from calf cerebral cortex which have chromatin of different repeat lengths. More H1° was found in the nuclei with the shorter repeat length chromatin. These nuclei are also believed to be the more active in RNA synthesis of the two types. Thus H1° contrasts with the avian erythrocyte-specific histone H5 in that the latter is associated with both increased repeat length and suppression of RNA synthesis. Since the central globular domains of H1° and H5 are highly homologous, it is suggested that the non-homologous, extended 'tails' of H1° and H5 are crucial to the function of these molecules.

Histone H1° has been consistently found in mammalian tissues having low rates of proliferation (1-4). It has been suggested to have a suppressive role in cell replication/DNA synthesis (2,4).

H1° and the avian erythrocyte-specific histone H5 are structurally very similar in their central globular domains (5-9). Total amino acid analyses (10,11) indicate greater differences in the extended 'tails' flanking the central domain. H5 has been shown to be closely associated with chromatin condensation (12,13) and Allan et al. (14) have suggested that the tails are crucial in implementing such condensation. Thus, the question arises as to whether H1° functions in a similar manner. This was examined here by looking at H1° in two types of nuclei, neuronal and glial, in which the latter has more condensed, longer repeat length chromatin (15).

METHODS

Fresh calf brains were supplied by a local slaughterhouse and placed in 0.32 M sucrose on ice immediately after removal from the animal. Nuclei were fractionated by the method of Thompson (16) except that the purity of these

0006-291X/82/210078-05$1.00/0

fractions was improved by the addition of a third layer of sucrose (2.1 M) between the 1.8 M and 2.4 M layers. A fraction of predominantly 'neuronal' nuclei [called 'N_1', as per Thompson (16)] was obtained from the interface of the 2.1 M and 2.4 M sucrose, while a population of 'glial' nuclei of greater than 90% purity [called 'N_2'] was retrieved as a pellet at the bottom of the 2.4 M sucrose layer. Each fraction was resuspended and washed in 0.32 M sucrose. Phenyl methyl sulphonyl fluoride was used at all stages to inhibit proteolytic degradation.

Quantitative extraction of proteins from the nuclei was achieved by extraction 5 or more times with $HClO_4$ (5% w/v). Histones H1, H1^o and some HMG's were precipitated using acidified acetone (8 vols.) (17).

Electrophoresis of $HClO_4$ extracts was performed on polyacrylamide (15%) gels in sodium dodecyl sulphate (SDS) (0.1% w/v) (18) and also on acid-urea (2.5 M) polyacrylamide (20%) slab gels based on the method of Panyim and Chalkley (19). Acid-urea gels were stained in coomassie brilliant blue R250 (0.1% w/v) and subsequently using the silver staining method of Wray et al. (20). SDS gels were stained in Procion Navy MXRB (0.2% w/v), (21), the bands being quantified by scanning at 580 nm. Statistical analysis was carried out using the t-distribution.

RESULTS

'Neuronal' and 'glial' fractions were isolated from each of two preparations of calf brain and a glial fraction alone from a third. The degrees of contamination of each nuclei type with the other, as shown by phase contrast microscopy, are shown in Table 1. Statistical analysis of the data in Table 1 showed a significant difference between the values for H1^o/H1 to a level of 0.005. As the fractions were cross-contaminated to some extent, the real values for H1^o:H1 in (totally) pure fractions must differ by an even greater extent than the figures in Table 1 would suggest. Assuming the level of contamination shown in the Table, the adjusted figures for H1^o/H1 are 0.094 for glial and 0.154 for neuronal nuclei.

Analysis of the extracts on acid-urea gels showed the presence of two subfractions of H1^o in both populations of nuclei and in total brain nuclei, as has been found in other tissues examined (22).

No significant difference in values for H1^o/H1 was found in different brain preparations. Brain has been reported to have only minimal endogenous protease activity (23) but to ensure no degradation occurred, protease inhibitor was used throughout the isolation of nuclei. The extracts were analysed on two gel systems (SDS and acid-urea) for signs of degradation. No trace of H1 degradation products [e.g. HMG8, (24)] was seen on either system.

Table 1. Ratios of $H1^{o}$:H1 in different fractions of nuclei from calf cerebral cortex.

Fraction	% Composition Neuronal	Glial	n	$H1^{o}$/H1 (S.D.)
"Neuronal" N_1	70%	30%	4	0.135 (0.006)
"Glial" N_2	10%	90%	6	0.099 (0.004)

n = no. of gels, the figure for each gel being the average of at least 2 scans.

Hence it does not seem possible that the higher $H1^{o}$:H1 ratio for neuronal nuclei is due to any loss of H1 during nuclei isolation. In support of this, comparison of $H1^{o}$:H1 ratios between extracts of ox liver nuclei and whole ox liver show no preferential loss of either protein (data not shown). Neither was there any sign of the putative $H1^{o}$ degradation product $H1^{o}$ c (10) on either gel system. Since phosphorylation is thought to be important in the function of H5 (see review, 25), acid-urea gels were stained with silver to check for the presence of phosphorylated forms of $H1^{o}$ (26). None was evident in any sample, thus phosphorylation is assumed not to affect the results presented here.

DISCUSSION

The results show the presence of $H1^{o}$ in both neuronal and glial nuclei but the neurones have significantly more of this protein, relative to H1, than do glia. Clearly then the $H1^{o}$ content of any one organ only represents the sum of those of each constituent cell type.

The presence of $H1^{o}$ in significant amounts in glia contrasts with the results of other workers (27) who detected $H1^{o}$ by immunofluorescence in neuronal cells but not in glial cells excepting those associated with the optic tract. If these few cells were to contribute all the $H1^{o}$ of the glial fraction, their $H1^{o}$ content would have to be very high and the difference between neuronal cells and the majority of glial cells would be still greater than was estimated here. Alternatively, $H1^{o}$ in the "non-optic tract" glial cells may have been inaccessible to the antibody used by those workers.

In a study to be reported elsewhere, calf brain neuronal nuclei and glial nuclei had repeat lengths of 165.4 and 193 base pairs respectively. These figures are in reasonable agreement with the sizes reported for the corresponding nuclei from rabbit brain (15). Thus the present work shows $H1^{o}$ to be more prevalent in the cell population possessing the shorter repeat length chromatin. This contrasts with the reported association of H5 with increased repeat length (12,13).

In avian erythrocytes the appearance of H5 (in phosphorylated form) correlates well with suppression of RNA synthesis. Thus H5 is thought to be instrumental in that suppression (see review 25). Our results do not support such a suppressive role for $H1^{o}$ since it has proved to be more prevalent in the neuronal fraction than in the glial and Thomas and Thompson (15) found the RNA synthesis rate in similarly prepared neuronal fractions from rabbit cerebral cortex to be three times higher than in the corresponding glial fractions. However, neither do we infer from our results that $H1^{o}$ stimulates RNA synthesis since two other reports go against such an idea. Keppel et al. (28) interpreted their results as suggesting a role for IP25 (an apparently identical protein to $H1^{o}$) in suppression of RNA synthesis, while Smith and Johns (29) found no difference in $H1^{o}$ levels between bulk chromatin and mononucleosomes putatively enriched in transcribing sequences. Thus, taking all results together, we assume that $H1^{o}$ has no effect on RNA synthesis.

Since DNA synthesis occurs in the brain at least during early post-natal stages (30) the possibility exists that DNA synthesis is occurring at different levels in the two cell types and that the different $H1^{o}$/H1 values are a reflection of this.

In conclusion, $H1^{o}$ and H5 appear to behave differently from one another both in their association with chromatin of differing repeat lengths and with their correlation with RNA synthesis. These differences may reflect the importance of the 'tail' regions of these molecules (see introduction). Thus we suggest that while the highly homogeneous globular domains of $H1^{o}$ and H5 locate the proteins on similar positions on the nucleosome, the different

'tail' regions of the two molecules enable them to execute their particular (different) functions. This concept supports the hypothesis of Allan et al. (14) that the tail regions of histones are crucial in executing chromatin condensation.

ACKNOWLEDGMENTS

We thank all our colleagues, especially E.W. Johns, for their support. The work was funded by MRC grants.

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