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POSTNATAL APPEARANCE OF A SHORT DNA REPEAT LENGTH IN NEURONS OF THE CEREBRAL CORTEX

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SUMMARY A short DNA repeat length of 162 base pairs was present in neurons of the adult rabbit cerebral cortex while a 198 base pair repeat was found in cortical glial and kidneycells. The short DNA repeat length in cortical neurons was not evident in fetal or new born brain but it appeared during early postnatal development between 2 1/2 and 3 1/2 days in rabbit and between 4 and 7 days in mouse.

Nucleosomes are the fundamental repeating units in eukaryotic chromatin. The DNA repeat length of chromatin has been found to vary depending on the organism and the tissue apparently due to differences in the length of DNA in the linker region between nucleosome core particles (1,2). All eukaryotes which have been studied have 140 base pairs (b.p.[1]) of DNA associated with the core particle. Several tissues in various higher organisms exhibit DNA repeat lengths of close to 200 b.p. while actively dividing tissue culture cells generally yield values 10-15 b.p. lower (1,3). Thomas and Thompson have reported that nuclei from two mature cell types within the same mammalian tissue have widely divergent DNA repeat lengths (4). They found that neuronal nuclei isolated from the cerebral cortex have a repeat length of 160 b.p. whereas cortical glial nuclei demonstrated a 200 b.p. repeat. Given this pronounced difference it was of interest to determine whether the DNA repeat length in cortical neurons changed during maturation of the cerebral cortex. It was found that the short DNA repeat length was not present in cortical neurons at stages immediately after birth. Analysis of nuclei isolated from total cerebral cortex and from cortical neuronal perikaryon revealed that a 198 b.p. DNA repeat was replaced by a 162 b.p. repeat between 2 1/2 and 3 1/2

[1]Abbreviation: b.p., base pairs.

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days after birth in rabbit brain development and between 4 and 7 days in mouse brain development.

MATERIALS AND METHODS

Brain and kidney nuclei were purified from white rabbits and white mice by sedimentation through dense sucrose (5). Total cerebral cortex nuclei were subfractionated into 'neuronal' and 'nonastrocytic glial' populations (6). Nuclei were also isolated (5) from neuronal perikaryon purified from rabbit cerebral cortex (7). Purified nuclei were digested for 2 min at 37°C with micrococcal nuclease at 125 units/ml and DNA isolated as previously described (8) except that nuclei were also digested with 20 µg/ml of DNase free RNase A for 15 min at 37°C. Phage T7+ DNA was digested with Hind II restriction endonuclease (9). DNA samples (20 µg or 10 µg for phage DNA) were subjected to electrophoresis on 2·5% polyacrylamide-0·5% agarose slab gels (10) as modified by Thomas and Furber (11). Gels were prerun for 1 hr and then run for 4 hr at 80 volts at 23°C. Following staining with 20 ug/ml ethidium bromide for 15 min, the gels were photographed under short wavelength ultraviolet light. Negatives were scanned at 560 nm in a Gilford spectrophotometer for calibration of migration distances.

RESULTS

Neuronal and nonastrocytic glial nuclei were isolated from the cerebral cortex of young adult rabbits and following digestion of the nuclei with micrococcal nuclease, the resultant DNA fragments were analyzed by electrophoresis on slab gels. Fig. 1 shows that DNA bands from cortical neuronal nuclei are clearly out of phase and contain shorter fragments compared to corresponding kidney DNA bands as multiples of increasing size are compared (Fig. 1B). In contrast DNA bands fromcortical glial nuclei remained in phase with kidney bands (Fig. 1C). The average length of the DNA fragments in each of the bands in Figs. 1B and 1C was determined by parallel electrophoresis of phage T7+ DNA fragments of known length which had been generated by digestion with Hind II restriction endonuclease (Fig. 1A). A calibration curve was obtained by plotting the known lengths of these phage DNA fragments (12) against their relative mobilities (Fig. 2). DNA repeat lengths were determined as shown in Fig. 3 by plotting DNA fragment length in base pairs against band number (11, 13). Cortical glial and kidney nuclei both demonstrated DNA repeat lengths of 198 $\pm$ 4 b.p. while cortical neuronal nuclei had a distinctly shorter repeat length of 162 $\pm$ 4 b.p. (average of 8 independent isolations). Doubling or decreasing the nuclease concentration by half did not affect the DNA repeat

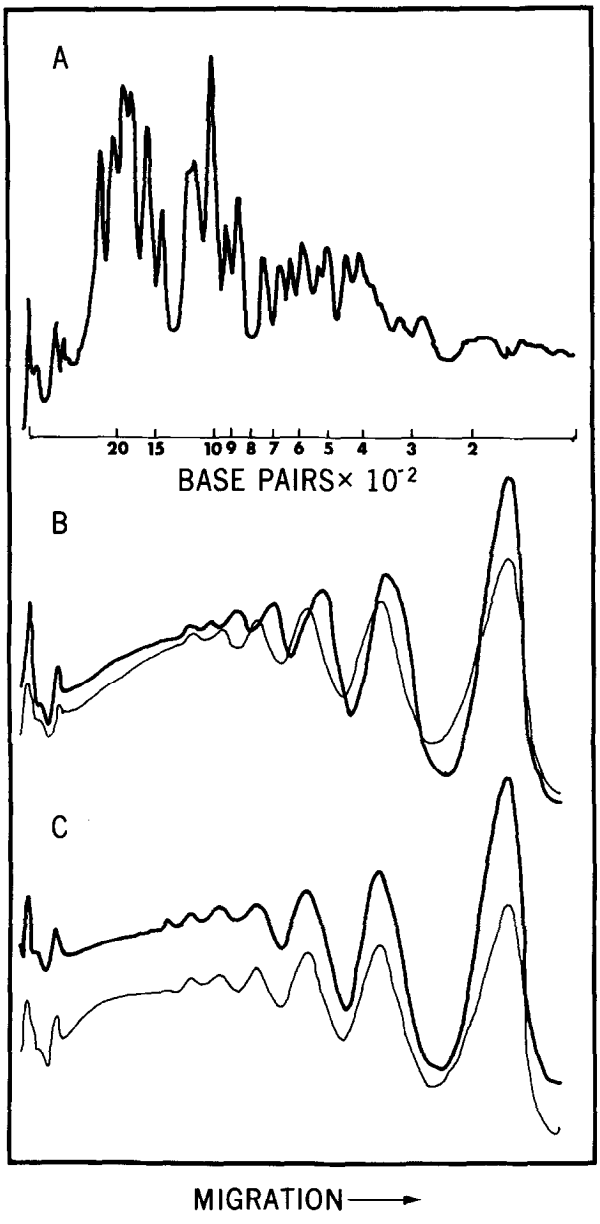

Fig. 1 Polyacrylamide gel electrophoresis of DNA fragments after micrococcal nuclease digestion of nuclei from young adult rabbit. A) marker DNA- Hind II digest of phage T7+ DNA; B) neuronal DNA (▬), kidney DNA (-): C) glial DNA (▬), kidney DNA (-).

length values. Since all nuclei exhibited a 140 b.p. fragment associated with the nucleosome core particle (fastest migrating band in Figs. 1B and 1C), the

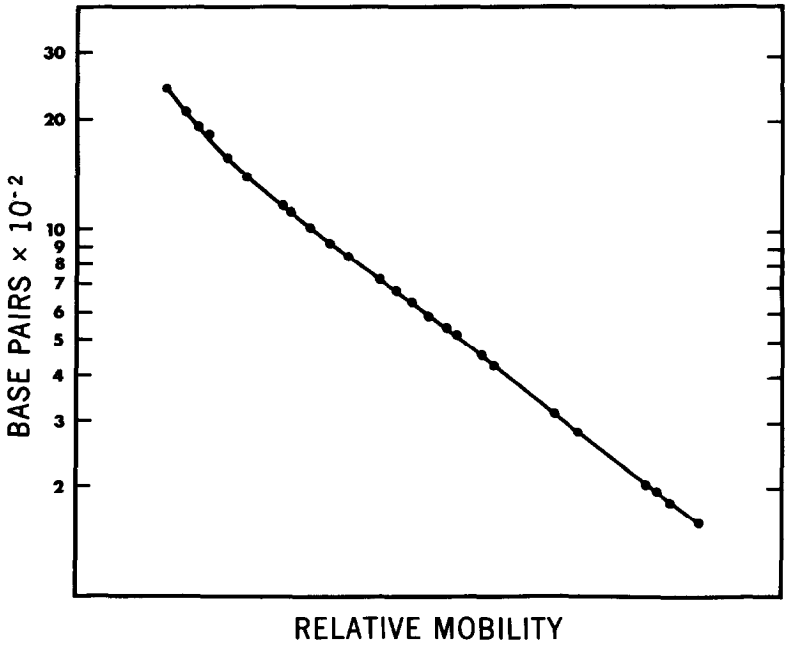

Fig. 2 Calibration curve of phage T7+ DNA fragments. Phage DNA was digested with Hind II restriction endonuclease and subjected to electrophoresis as in Fig. 1A. Size of restriction fragments is as given by Ludwig and Summers (12).

short repeat length in cortical neurons appeared to be the result of a reduced length of DNA in the linker region of the nucleosome (4).

Total cerebral cortex nuclei were isolated from rabbit brain at various stages of development and digested with micrococcal nuclease to determine whether DNA repeat length of chromatin changed during maturation of cortical cells. Nuclei from the cerebral cortex were not fractionated into neuronal and glial populations because of difficulties in separating these two nuclear types at early stages of brain development. Since the number of neurons in the rabbit cerebral cortex exceeds glial cells by a ratio of 2·5 to 1 (4, 14), a shift in the direction of a shorter DNA repeat length should be detectable in the summation pattern of DNA fragments generated from unfractionated cerebral cortex nuclei. As shown in Fig. 4 the DNA bands for total cerebral cortex nuclei were in phase with corresponding kidney bands at the following developmental stages- fetal (F), newborn (NB) and 2 1/2 days postnatal. By 3 1/2 days

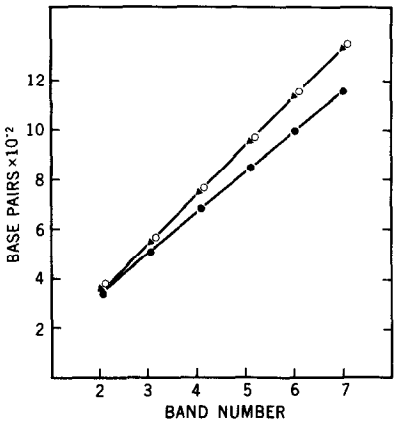

Fig. 3 Determination of DNA repeat length in neuronal (•), glial (o) and kidney (▲) nuclei isolated from young adult rabbits.

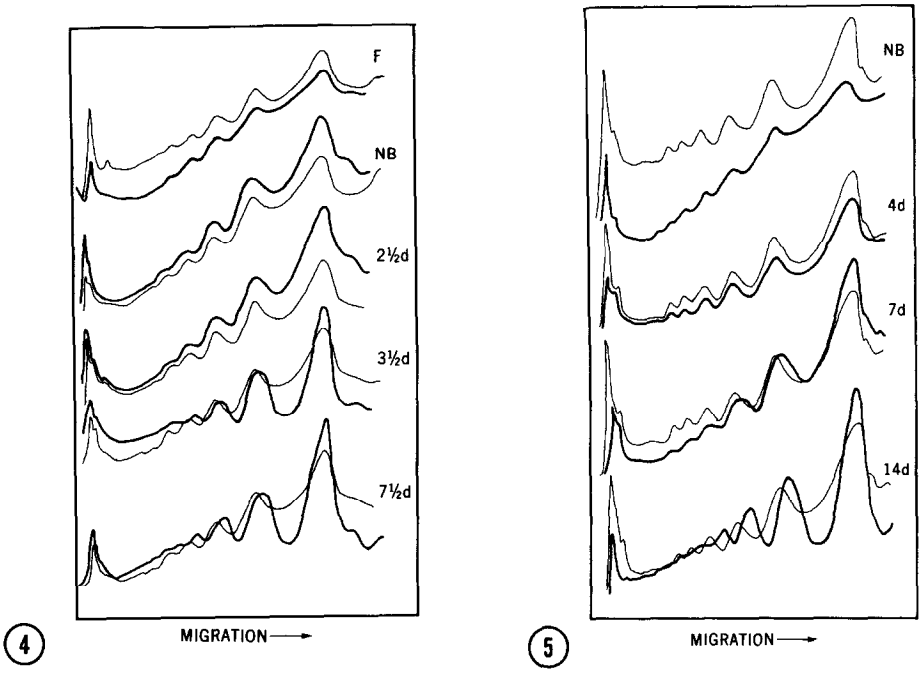

Fig. 4 Appearance of a short DNA repeat length during early postnatal development of the rabbit cerebral cortex. Nuclei isolated from cerebral cortex (▬) or kidney (-) at the indicated stages of development were digested with micrococcal nuclease and the resultant DNA fragments were analyzed by slab gel electrophoresis.

Fig. 5 Change in DNA repeat length during early postnatal development of the mouse cerebral cortex. Cerebral cortex DNA (▬); kidney DNA (-).

the cortical DNA bands were out of phase and contained shorter DNA fragments than corresponding kidney bands as multiples of increasing size were compared. This difference in rabbit cortical DNA bands was further accentuated by 7 1/2 days of postnatal development. In the mouse cerebral cortex no evidence of a short DNA repeat length was seen in newborn or 4 day postnatal animals (Fig. 5). A shift to shorter DNA fragments was, however, observed at 7 days and further accentuated by 14 days. While the gel scans in Figs. 4 and 5 suggested that there was a developmental change to a shorter DNA repeat length as the cortical cells matured, the pattern of kidney DNA fragments remained the same.

To verify that the shift towards a shorter DNA repeat length, which was observed in cortical nuclei, really reflected a developmental alteration in chromatin in neuronal cells, neuronal perikaryon (i.e. neuronal cell bodies with axons sheared off) were isolated from rabbit cerebral cortex. By phase contrast microscopy the neuronal perikaryon preparation was free of glial cell contamination. Nuclei purified from the isolated perikaryon were digested with micrococcal nuclease and the DNA repeat length was determined as in Fig. 2. Nuclei from perikaryon isolated 1 1/2 days after birth exhibited a DNA repeat length of 198 $\pm$ 6 b.p. while a short repeat of 162 $\pm$ 7 b.p. was evident in perikaryon isolated 5 1/2 days after birth (average of 4 independent isolations). These data together with the data given in Figs. 4 and 5 suggest that a short DNA repeat length appeared postnatally in cortical neurons between 2 1/2 and 3 1/2 days in the rabbit and between 4 and 7 days in the mouse.

DISCUSSION

These results indicate that cortical neuronal cells undergo a postnatal developmental change in chromatin conformation. In rabbit brain a DNA repeat length of 198 b.p. was present in cortical neurons 1 1/2 days after birth. The conversion to a short repeat length commenced between 2 1/2 and 3 1/2 days after birth and appeared complete by 5 1/2 days when the adult neuronal repeat of 162 b.p. was attained. A postnatal decrease in the repeat length of cortical neurons was also observed during development of the mouse brain. In mouse the

conversion commenced between 4 and 7 after birth. Since cell division does not occur in cortical neurons during postnatal development the change in DNA repeat length appeared to be independent of mitosis.

At all stages of cortical neuronal development in both the rabbit and mouse a 140 b.p. DNA fragment was observed associated with the nucleosome core. This suggested that the short DNA repeat of 162 b.p. was associated with a short length of DNA in the linker region of the nucleosome i.e. 22 b.p. after the conversion compared to 58 b.p. at very early stages of development. In contrast the linker region of kidney nucleosomes remained unchanged at all stages. The short DNA repeat lengths of 154-170 b.p. reported for a number of lower eukaryotes also appears to be associated with short lengths of DNA in the linker region (1, 15).

The developmental importance of the early postnatal change to a short DNA repeat length remains unknown. Thomas and Thompson, who first reported the short neuronal repeat length, suggested that it might be correlated with the high RNA synthesis activity which they observed in isolated cortical neuronal nuclei, however, they advised caution in this interpretation (4). We have previously noted that the transcription of nonrepeated DNA in total mouse brain increased postnatally (16). It should be pointed out, however, that the transcription of nonrepeated DNA in the cerebral cortex and the cerebellum is similar (17) yet the short repeat is not present in the cerebellum (4).

The functional significance of the core and linker regions of the nucleosome is unknown at present. This system in which the nucleosome structure changes developmentally may be useful in order to approach a basic functional analysis. The fact that the change occurs in cortical neurons in the brain is of particular interest given the complex neural functions that these cells undertake as development proceeds.

NOTE A partial report of this study was presented at a satellite symposium of the 6th International Meeting of the International Society for Neurochemistry, Amsterdam, Aug. 29-31, 1977.

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of the manuscript. Phage T7+ DNA and Hind II restriction endonuclease were gifts from Dr. Paul Sadowski. This study was supported by grants from the National Research Council of Canada.

Note Added in Proof: It has recently been reported that the DNA repeat length in rat cortical neurons is 195 base pairs in the fetus (2 days before birth) and 174 base pairs in the infant (7 days after birth) and in the adult. Ermini, M. and Kuenzle, C.C. (1978) FEBS Lett. 90, 167-172.

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