THE CHROMATIN REPEAT LENGTH OF CORTICAL NEURONS SHORTENS DURING EARLY POSTNATAL DEVELOPMENT

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1. Introduction

Work from this laboratory has given evidence for an elevated DNA content in cortical neurons of various mammals [1,2] including man (A. Bregnard and C. C. K., unpublished). The developmental course of this phenomenon has been followed in the rat. This revealed a rapid DNA increase from a 2c DNA complement in near-term fetuses (c, DNA content of haploid chromosome set) to a 3c level at 7 days postnatal age followed by a further slow rise to 3.5c during the next 3 weeks [1,2].

A reduced chromatin repeat length of approx. 160 base pairs of DNA in cortical neurons from adult rabbits and rats, as compared with about 200 base pairs for nonastrocytic glial cells, cerebellar neurons and liver has been described [3]. (For a recent review on chromatin structure, see [4].) It thus occurred to us that a temporal link might exist between this shortening of the chromatin repeat length and the DNA increase in cortical neurons described in the preceding paragraph.

The present study was undertaken to resolve this question. Neuronal nuclei from the cerebral cortex and liver nuclei were isolated from rats of various developmental stages (2 days before birth, and 7, 30 and 60 days postnatal age).

Electrophoretic analysis of the DNA fragments resulting from partial digestion with micrococcal nuclease revealed an average DNA repeat length of 195 ± 8 base pairs for fetal and of 174 ± 3 base pairs for infant and adult neuronal chromatin. This shortening of the DNA repeat length takes place within the first postnatal week thus showing a close temporal correspondance with the increase in DNA content. Additional experiments established that the shortening is confined to the internucleosomal linker DNA, whereas the core DNA retains a length of 140 base pairs at all developmental stages.

In contrast to the neurons, liver nuclei undergo lengthening of the chromatin repeat from 185 ± 3 base pairs in fetuses to 207 ± 4 base pairs in adult rats.

2. Materials and methods

2.1. *Animals*

SIV-50 rats of either sex were used at the following developmental stages: 2 days before birth (–2 d), 7 days (7 d), 30 days (30 d) and 60 days (60 d) after birth.

2.2. *Preparation of nuclei*

Nuclei from cerebral cortex neurons were isolated over 90% pure from either the entire cerebral hemispheres (fetuses) or from cortex (postnatal stages) according to the method described previously [5,6].

Nuclei from liver were prepared as in [7].

Immediately after preparation the nuclei were washed 3 times in a solution containing 15 mM Tris–HCl, pH 7.4, 15 mM NaCl, 60 mM KCl, 15 mM β-mercaptoethanol, 0.5 mM spermidine, 0.15 mM spermine [8] and counted in a pulse cytophotometer (ICP-11, Phywe, Göttingen) as in [9].

2.3. *Nuclease digestions*

Nuclei were digested with either micrococcal

nuclease (Worthington) as in [10] or pancreatic DNase I (Worthington) as in [11] with 75 U enzyme/10^8 nuclei at 37°C for the times required to solubilize 7–15% of total DNA (400–650 s for neuronal and 200–450 s for liver nuclei).

2.4. *DNA extractions*

After incubation the nuclei were directly extracted in 1 M NaCl, 1% SDS with isoamylalcohol–chloroform (1:24). DNA was precipitated from the aqueous phase with 95% ethanol at −20°C [11].

2.5. *Gel electrophoresis and base-pair determination of the DNA fragments*

Double-stranded DNA was electrophoresed on 0.5% agarose–2.5% polyacrylamide slab gels (0.2 × 15 × 17 cm) as in [12] at 40 mA for 2.5 h.

Single-stranded DNA was analyzed on 7 M urea–12% polyacrylamide slab gels (0.2 × 15 × 20 cm) as in [13].

After staining with ethidium bromide the gels were photographed under short-wavelength ultraviolet light. Base-pair determinations were performed as indicated in table 1 and fig.2.

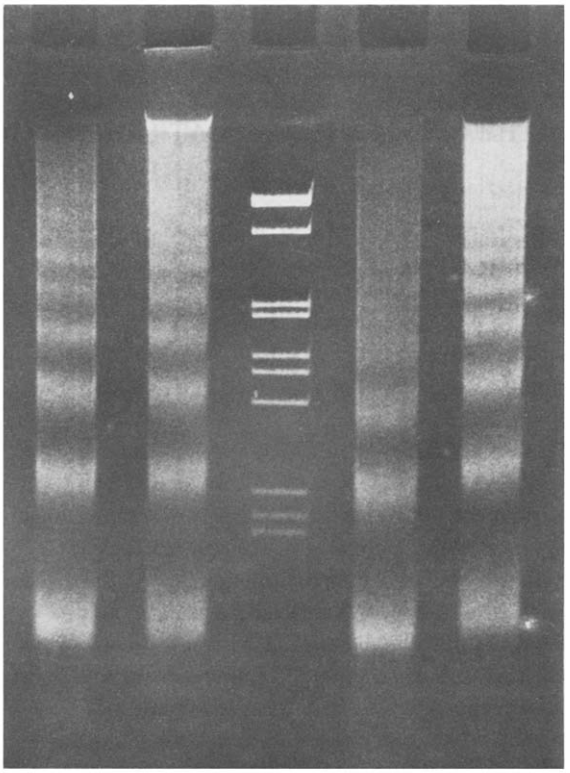

Fig.1. Micrococcal nuclease digests of DNA from cerebral cortex neurons (N) and liver (L) of fetal (−2 d) and adult (60 d) rats electrophoresed on a 0.5% agarose–2.5% polyacrylamide slab gel. Hae III digested PM2 DNA was run as a marker. Differences in mobility are particularly well visible between 60 d N and 60 d N.

Table 1
Average repeat lengths (base pairs) of nucleosomal DNA from cerebral cortex neurons (N) and liver (L) of fetal (−2 day) and adult (60 day) rats

	Base pairs			
	−2 day N	60 day N	−2 day L	60 day L
bp/*n* (± SD)	195 (±8)	174 (±3)	185 (±3)	207 (±4)
Δbp (± SD)	215 (±20)	179 (±8)	182 (±18)	210 (±15)

The base pair numbers of oligomers 2–8 (adult neurons, 2–6) were either divided by their respective band number (bp/*n*) or subtracted consecutively from each other (Δbp) for computation of the monomer repeat lengths. The data were taken from three independent gels. The base pair content of the DNA oligomers was determined on calibration curves obtained from Hae III digested PM2 fragments [15], which were electrophoresed simultaneously with the micrococcal nuclease digests (see also fig.1)

3. Results

An electrophoretic comparison of the DNA fragments obtained from neuronal and liver nuclei after partial digestion with micrococcal nuclease is shown in fig.1. Fetal DNA from both types of nuclei exhibit identical patterns. In contrast, at 60 days postnatal age all neuronal DNA fragments show increased mobilities not only with respect to 60 day old liver but also to fetal nuclei of both cell types.

The average repeat lengths of the various types of chromatin investigated are presented in table 1. It is evident that in neurons the nucleosomal DNA shortens from approx. 195 base pairs in fetuses to 174 base pairs in adults, while in liver concomitant lengthening occurs from 185–207 base pairs. For liver a similar developmental lengthening has been observed [14].

Plotting the average lengths of DNA fragments versus the respective band numbers gave the regression lines shown in fig.2. It can be seen that both for neurons and liver the regression lines representing fetal and adult stages, respectively, diverge from a common point of origin on the ordinate and are not simply displaced parallel to each other. This indicates true variations in repeat lengths rather than terminal trimming as a result of, e.g., variable exonucleolytic activity [3]. This conclusion is further supported by the analyses represented in fig.3a and 3b, since no increase in mobility of individual bands can be seen as a result of increasing times of digestion.

Figure 4 shows the developmental course of the shortening of the chromatin repeat length in neurons. This process is clearly restricted to the first postnatal week. After that no further significant changes occur as evidenced by the parallel patterns displayed by the DNA fragments from neurons of age groups 7, 30 and 60 days.

To distinguish whether the described shortening of the chromatin repeat length is brought about by changes in either the linker or the core DNA, neuronal nuclei were subjected to prolonged incubation with micrococcal nuclease. The released monomeric DNA fragments were then analyzed by electrophoresis on denaturing gels and were compared with monomeric DNA fragments from similarly treated liver nuclei known to be cleaved into repeats of 140–160 bases [15]. Further calibration was performed with a pancreatic DNase I digest of neuronal chromatin previously shown to give the typical 10 base repeat pattern as observed with liver (unpublished observation and [11]). As shown in fig.5 monomeric DNA fragments from both fetal and adult neurons banded

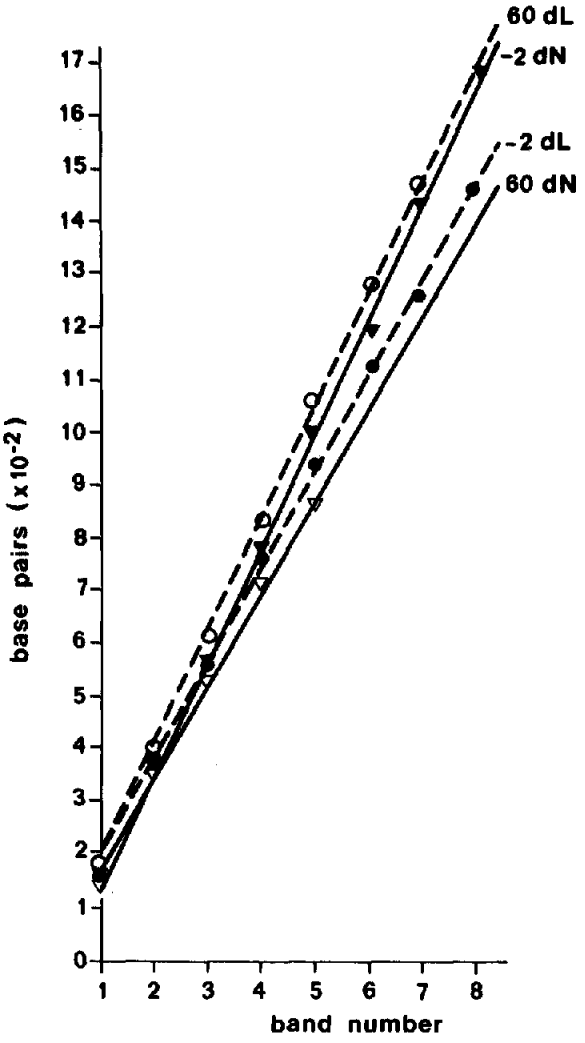

Fig.2. DNA lengths of nucleosomal oligomers after micrococcal nuclease digestion of nuclei from cerebral cortex neurons (N) and liver (L) of fetal (−2 d) and adult (60 d) rats. Base pair numbers (bp/*n*) were determined as indicated in table 1. For measurements of the distances of migration the photographs of the gels were scanned and the peak maxima taken as midpoints of the bands. Data were taken from 3 independent gels. The lines were obtained from linear regression calculations, r = 0.9996, 0.9990, 0.9993, and 0.9996 for −2 d N, 60 d N, −2 d L, and 60 d L, respectively.

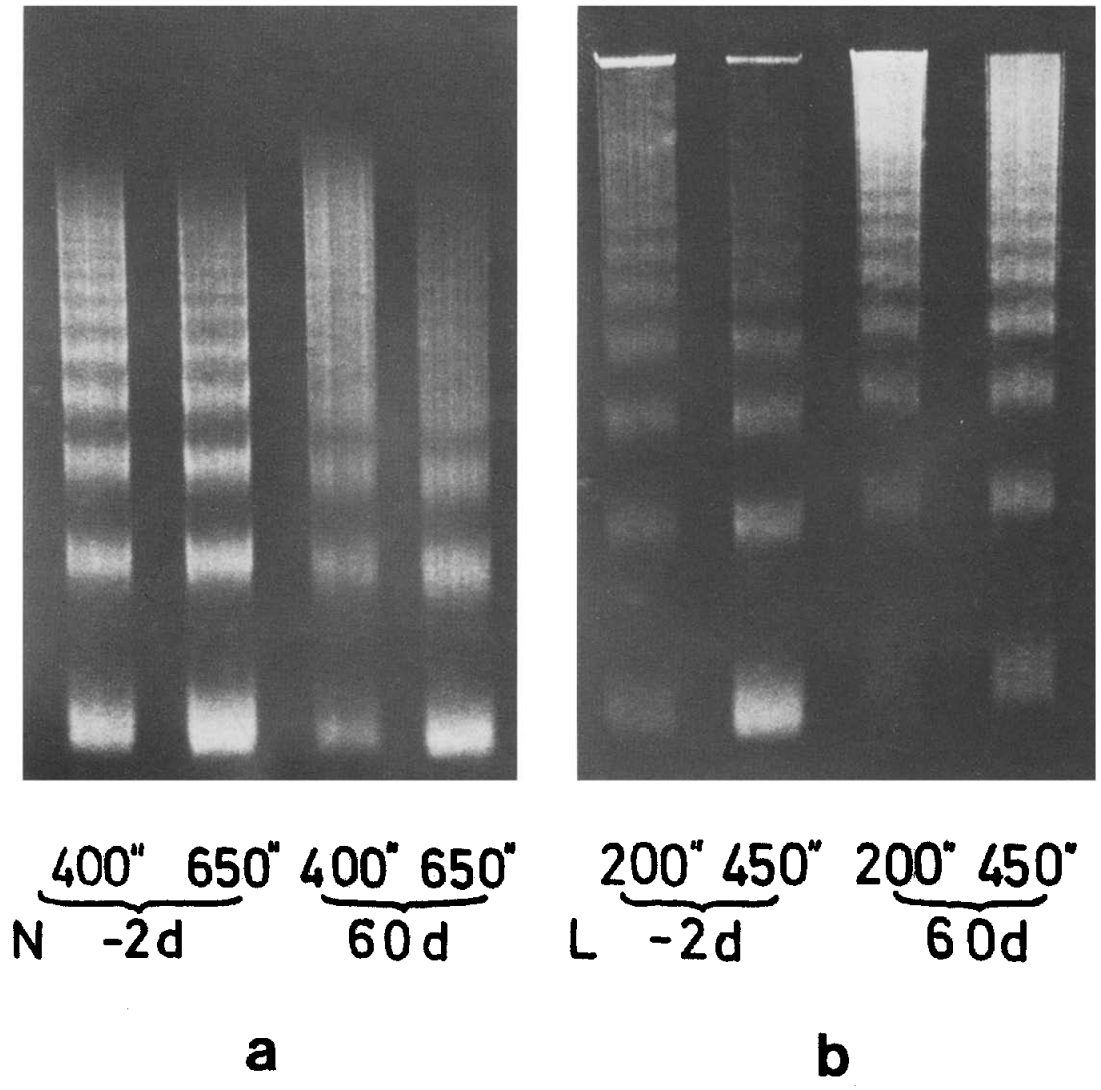

Fig.3. DNA digests of cerebral cortex neurons (N) and liver (L) of fetal (−2 d) and adult (60 d) rats after various times of incubation with micrococcal nuclease. Note that the mobilities of the DNA fragments are not affected by the two different incubation times.

at identical positions as liver monomers, and comparison with the pancreatic DNase I digest further established a repeat length of 140 bases for the nucleosomal core DNA. Thus, the shortening of the DNA repeat length of neuronal chromatin during development affects the linker region only, whereas the nucleosomal core remains unaltered. A similar conclusion has been reached [3].

4. Discussion

The results reported above demonstrate that the chromatin repeat length of rat forebrain cortical neurons shortens during early postnatal development. This change is temporally linked to a wave of replicative DNA synthesis resulting in a net increase of the nuclear DNA content of these neurons [1,2].

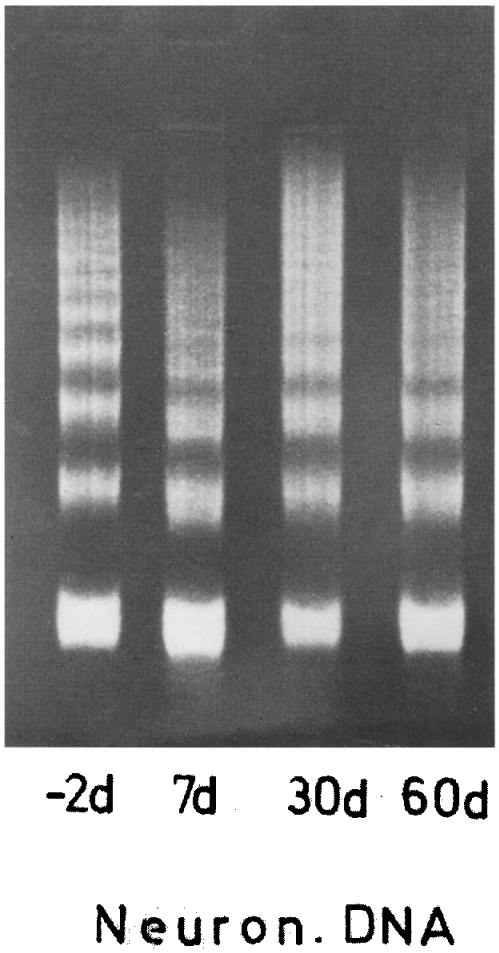

Fig.4. Comparison of micrococcal nuclease digests of DNA from cerebral cortex neurons (N) of rats of various ages (−2 d, 7 d, 30 d, 60 d) showing developmental shortening of the chromatin repeat length during the first postnatal week.

Whether a causal relationship exists between these two events is unclear at present, but in view of the findings [14], suggesting an inverse correlation between DNA replicative activity and chromatin repeat length for some mammalian cells, this assumption would not seem unreasonable. On the other hand, Thomas and Thompson [3], who first observed the shortened DNA repeat length in adult cortical neurons, tentatively ascribed this feature to the high transcriptional activity prevailing in these cells. This would be consistent with the increase in RNA synthetic activity as reported [16] for whole brain neuronal nuclei during the first 2 weeks of postnatal development. However, the opposite findings [17,18] claiming that RNA polymerase activity declines in cerebral neurons after birth caution against the unreserved acceptance of such a correlation. In addition, the study of a wide variety of cells and tissues [14] came to the conclusion that no general correlation exists between transcriptional activity and chromatin repeat length. Similarly, differences in repeat length between transcriptionally active and inactive rat liver chromatin could not be found [19].

In conclusion, though a clear temporal correspondance exists between the shortening of the chromatin repeat length and the DNA increase in cortical neurons a causal relationship remains to be established.

Acknowledgements

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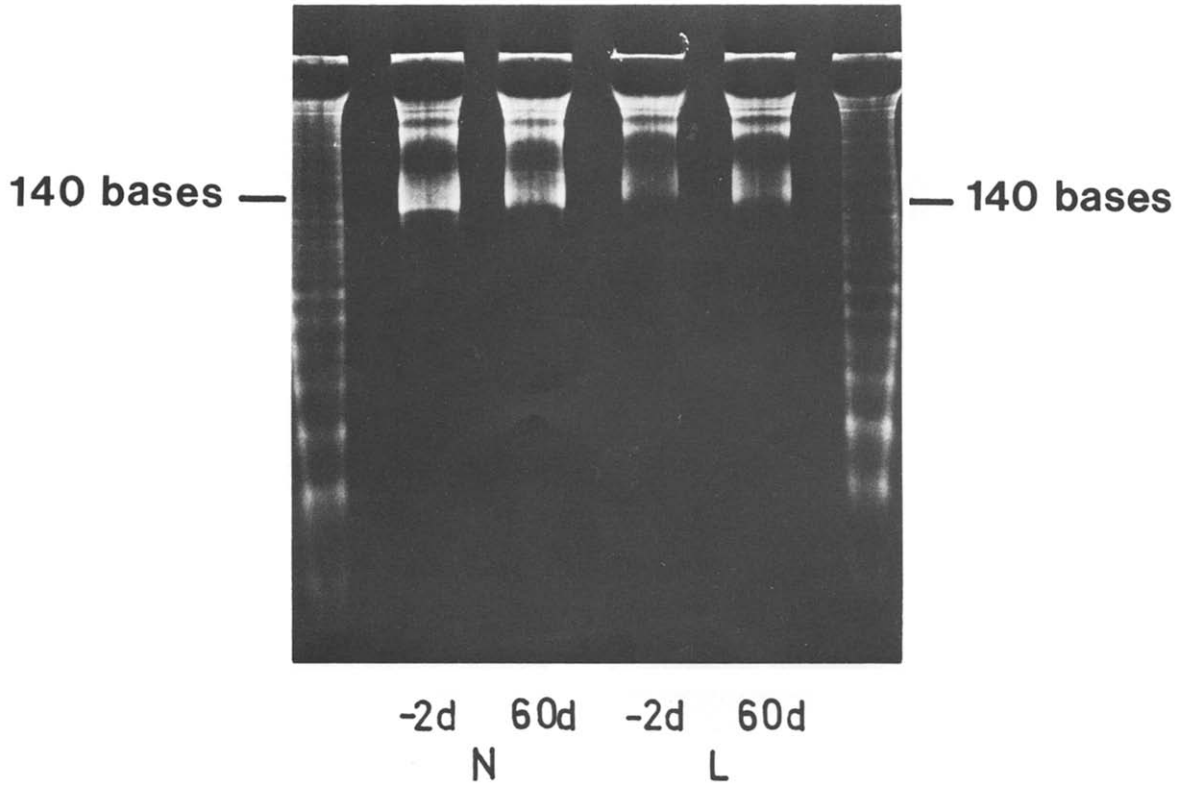

Fig.5. Determination of the number of base pairs within nucleosomal monomers obtained by prolonged micrococcal nuclease digestion of nuclei from cerebral cortex neurons (N) and liver (L) of fetal (−2 d) and adult (60 d) rats. DNA fragments were run on a 7 M urea-12% polyacrylamide gel in presence of a pancreatic DNase I digest of rat neuronal DNA.

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