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Methylation of repetitive DNA sequences in the brain during aging of the rat

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Methylation of repetitive DNA sequences (RDS) of the genomic DNA of the brain of 15- and 88-week old rats was analysed by digestion with *Hpa*II, *Msp*I, *Eco*RI + *Hpa*II and *Eco*RI + *Msp*I followed by end-labelling. $\overset{m}{C}$pG doublets are present in two RDS of ~ 5 and 0.4 kb, and are also randomly distributed throughout the genome. Hemimethylated $\overset{m}{C}$pC doublets also occur. Both $\overset{m}{C}$pG and $\overset{m}{C}$pC doublets are found more in the old than in the young. This age-related increase in DNA methylation occurs both at CCGG sites of the RDS and in the entire genome. Such increase in DNA methylation may alter chromatin conformation and gene expression in the brain as the rat ages.

DNA methylation; Restriction endonuclease; Repetitive DNA; Age-related change

1. INTRODUCTION

Three to ten percent of cytosines of the vertebrate DNA is methylated in a sequence-specific and tissue-specific manner [1–3]. DNA methylation has been implicated in the control of transcription [4–7], replication, transposition, DNA repair and chromosome configuration [1,8,9], as well as inheritance of specific patterns of gene activity [10,11]. Tissue-specific differences in the methylation of certain genes [5], highly repeated satellite DNA sequences [8,9,12] and whole genome [2,3] often do not correlate with transcriptional activity. Whether methylation is a primary signal for gene expression [13] or is a maintenance signal for patterns established by other mechanisms [14,15], is not clear. The role of specific *trans*-acting protein factors that recognise DNA sequences containing 5mC residues in the genome and modulate various functions of DNA is being increasingly recognised [11,16,17]. *trans*-acting factors may also modify the methylases or interfere with them [18].

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The present study reports the analysis of the genomic DNA of the brain of the rat by *Hpa*II, *Msp*I and *Eco*RI, and DNA methylation at CCGG sites during aging of the rat.

2. MATERIALS AND METHODS

Nuclei were purified from the brain of young (15 week) and old (88 week) Wistar albino female rats according to Burgoyne et al. [19]. DNA was extracted from nuclei according to Marmur [20] and Axel et al. [21], and estimated in 2 M NaCl/5 M urea [22]. 50 μg DNA in 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA was digested by *Hpa*II, *Msp*I, *Eco*RI, *Eco*RI + *Hpa*II and *Eco*RI + *Msp*I (4 U/μg DNA). Control (no enzyme) and standard (non-methylated λ DNA) sets were run in parallel. 5 μg of the restricted DNA was labelled at the 5′-recessed ends of C$\downarrow$CGG sites using 5′-[α-^{32}P]dCTP (3000 Ci/mmol) and *E. coli* DNA polymerase I (Klenow fragment) (DNA pol.I). DNA fragments (5 or 15 μg/lane) were resolved on 1% agarose gels, stained with ethidium bromide and photographed under UV light. The labelled DNA fragments were resolved on 1% agarose gel and autoradiographed for 7 days by exposing to X-ray films [23].

3. RESULTS AND DISCUSSION

Several repetitive DNA sequences (RDS) appear as bands after digestion of the genomic DNA of the brain of both young and old rats for 1 h and 6 h by *Eco*RI, *Hpa*II, *Msp*I, *Eco*RI + *Hpa*II and

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*Eco*RI + *Msp*I. DNA fragments of heterogeneous sizes appear as smear. They are restricted at sites that are randomly distributed throughout the genome (figs 1,2).

*Eco*RI produces two distinct bands of ~2.5 and 1.3 kb as well as five smaller bands between 0.4 and 0.1 kb. These represent RDS of long and short periodicities, respectively, that are flanked by G↓AATTC sites. The *Eco*RI digestion pattern is preserved even after *Eco*RI + *Hpa*II and *Eco*RI + *Msp*I digestions. *Hpa*II or *Msp*I alone produces random DNA fragments between 20–2 kb and 20–0.4 kb, respectively. Two RDS of ~5 and 0.4 kb flanked by C↓CGG sites appear after *Msp*I, but not after *Hpa*II digestion. Moreover, the extent of digestion by *Msp*I is far greater than that by *Hpa*II. Both *Hpa*II and *Msp*I cut non-methylated C↓CGG sequences. *Hpa*II cannot cut this sequence if the internal C is methylated, but *Msp*I can. So it appears that the genome of rat brain contains a

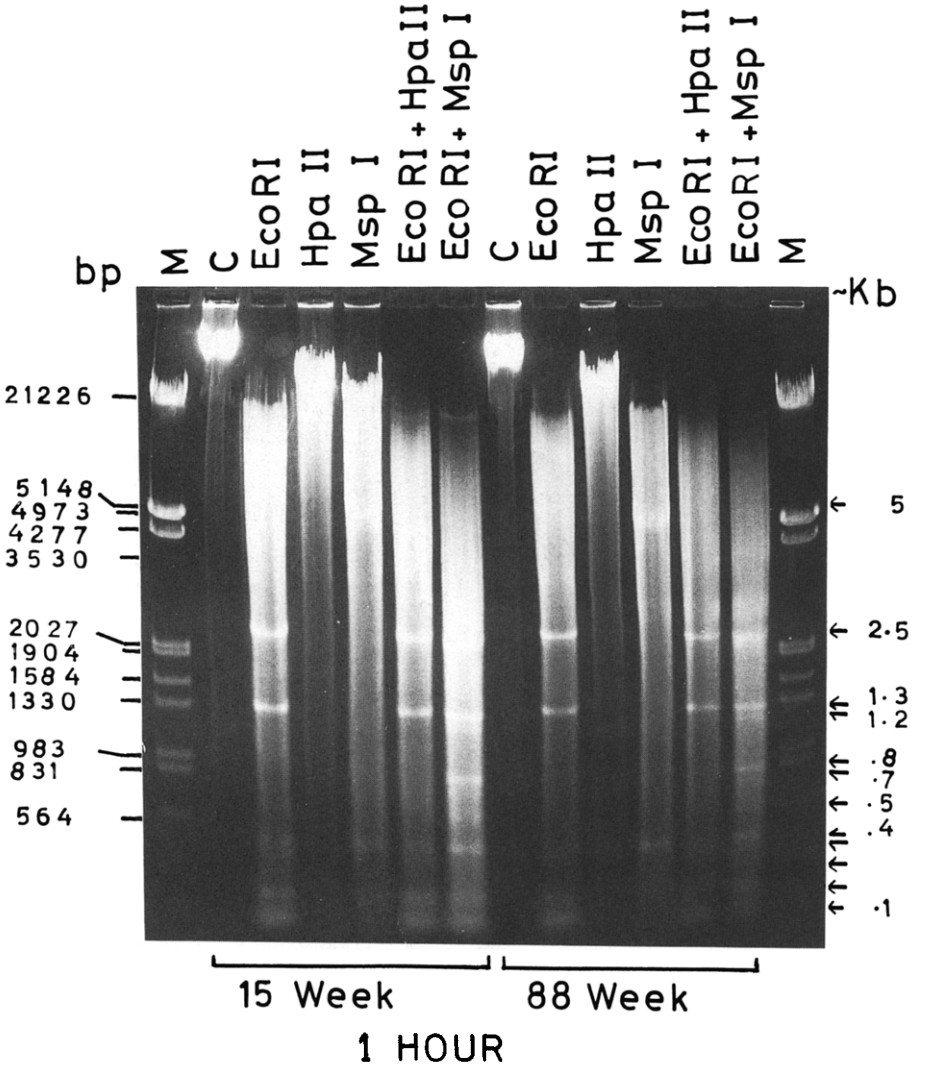

Fig.1. Genomic DNA restricted for 1 h. DNA of the brain of young (15 week) and old (88 week) female rats was digested by *Eco*RI, *Hpa*II, *Msp*I, *Eco*RI + *Hpa*II and *Eco*RI + *Msp*I for 1 h and the DNA fragments were resolved by 1% agarose gel. C, control (no enzyme); M, λDNA digested by *Eco*RI + *Hin*dIII; bp, base pair; kb, kilo base pair; ⟶ indicates repetitive DNA. 15 μg DNA was loaded per lane.

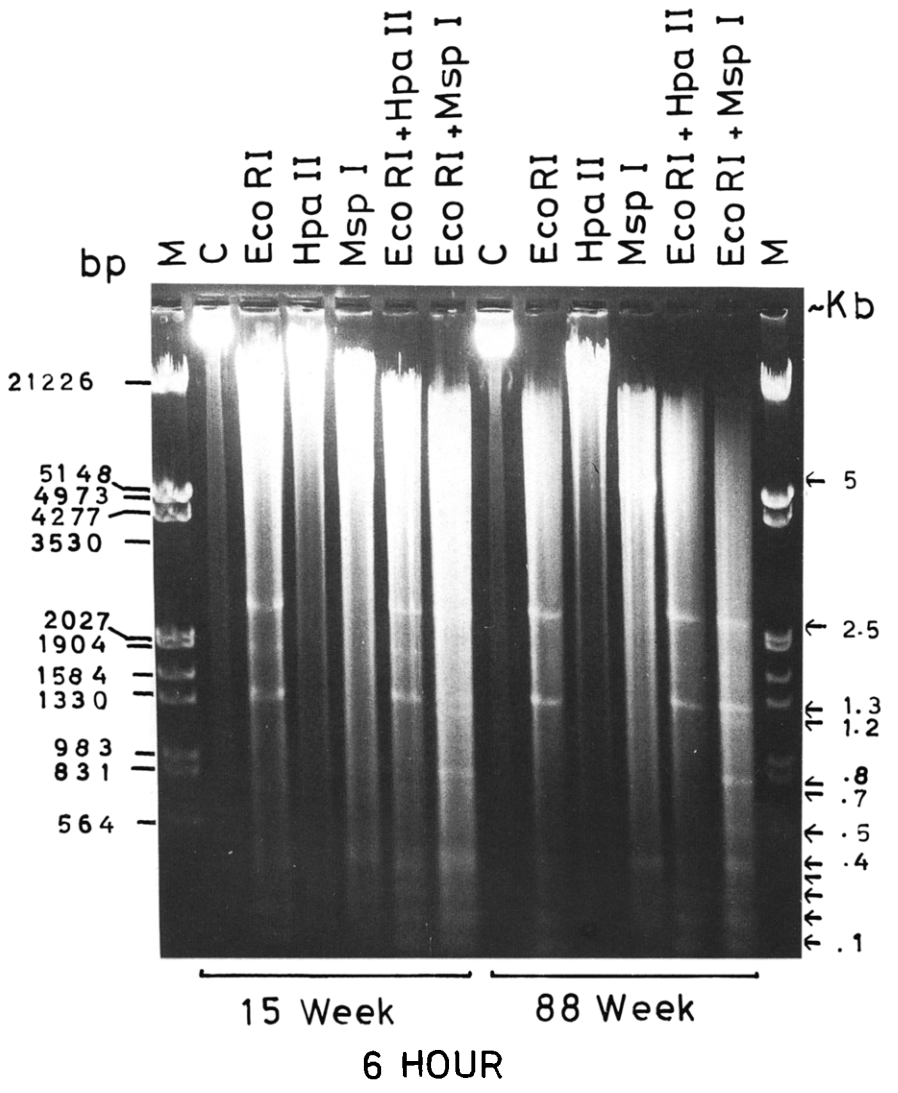

Fig.2. Genomic DNA restricted for 6 h (see legend for fig.1).

large number of 5mC residues at randomly distributed C$\overset{m}{C}$GG sites.

*Eco*RI + *Msp*I produce five DNA fragments of ~1.2, 0.8, 0.7, 0.5 and 0.4 kb which are not observed after *Eco*RI + *Hpa*II digestion. These RDS, therefore, have C$\overset{m}{C}$GG sites. They (except the 0.4 kb band) contain a G$^{\downarrow}$AATTC sequence at one end and a C$^{\downarrow}\overset{m}{C}$GG sequence at the other, because *Hpa*II, *Msp*I and *Eco*RI individually, and *Eco*RI + *Hpa*II cannot produce these fragments. Interestingly, the 5 kb *Msp*I band containing $\overset{m}{C}$pG doublet is not observed after *Eco*RI + *Msp*I digestion. Hence, it contains internal G$^{\downarrow}$AATTC sequences. This indicates that the additional fragments derived after *Eco*RI + *Msp*I digestion are generated from the 5 kb C$^{\downarrow}\overset{m}{C}$GG repetitive DNA.

The end-labelling of the restricted DNA fragments shows that the 5′-recessed ends produced after *Eco*RI digestion (G$^{\downarrow}$AATTC) do not incorporate any ^{32}P-dCMP (in the presence of DNA pol.I) (fig.3). However, the 5′-recessed ends (C$^{\downarrow}$CGG) produced after *Hpa*II, *Msp*I, *Eco*RI + *Hpa*II and *Eco*RI + *Msp*I digestions, incorporate

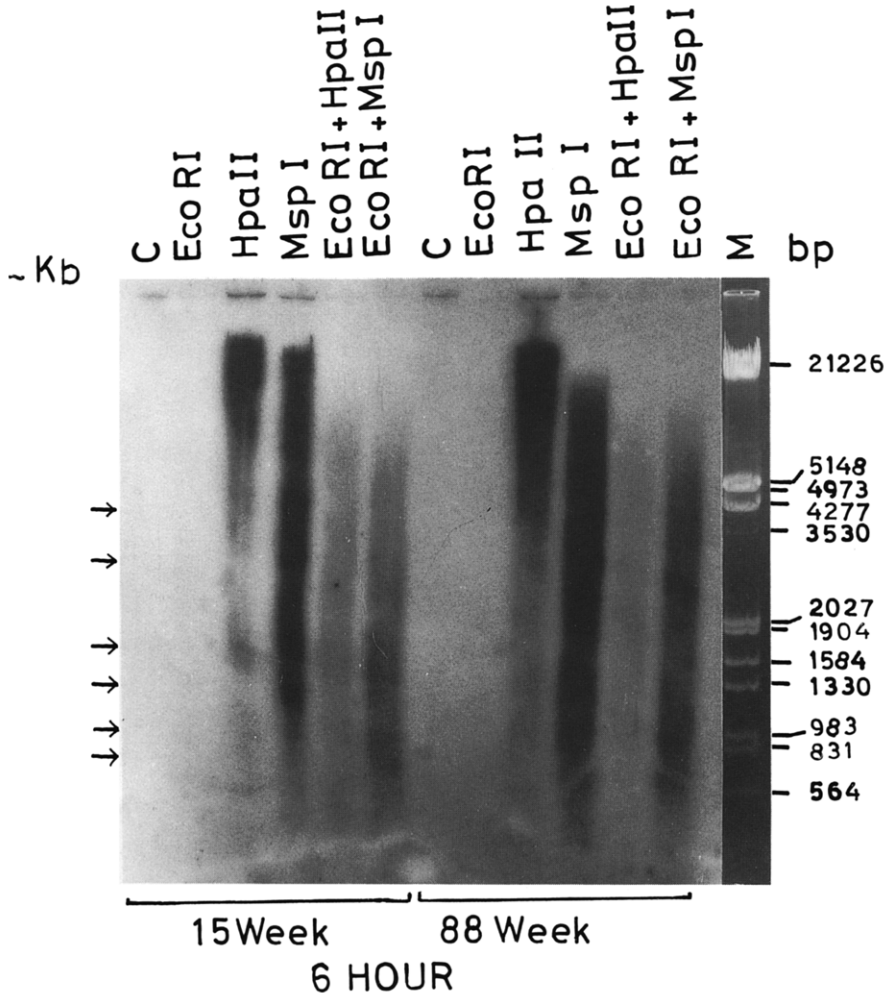

Fig.3. Autoradiogram of the gel shown in fig.2. 5 μg of the ^{32}P end-labelled restricted DNA fragments was loaded per lane. ⟶ indicates repetitive DNA fragments containing $\overset{m}{C}$pG doublets.

^{32}P-dCMP to fill the internal cytosines. Thus the end-labelling reaction is specific for C$^{\downarrow}$CGG sites cut by *Hpa*II or *Msp*I.

A larger number of DNA fragments are produced in the high molecular size-range in the old rats after *Hpa*II digestion. A fraction of these DNA fragments in the 20 kb size-range is produced in aged rats after *Hpa*II, but not after *Msp*I digestion. In the young, these DNA fragments are produced both by *Msp*I and *Hpa*II. *Msp*I cleaves C$^{\downarrow}$CGG, and also C$^{\downarrow}\overset{m}{C}$GG and $\overset{m}{C}^{\downarrow}$CGG if both strands are methylated. It cannot cleave $\overset{m}{C}^{\downarrow}$CGG if hemimethylated. In contrast, *Hpa*II can cleave this hemimethylated sequence containing external 5mC on one strand. It cannot cut the sequence if both the strands are methylated either at external or at internal cytosine. Therefore, some of the CCGG sites are hemimethylated at $\overset{m}{C}$pC sites and are cleaved by *Hpa*II. With age, these sequences may get fully methylated.

Both the extent of digestion and the amount of DNA fragments produced by *Msp*I are higher in old rats. Most of these CCGG sites are not cleaved by *Hpa*II (fig.3). Hence, these fragments have CpG doublets. They represent both repetitive and random distribution of CCGG sequences in the genome. Therefore, it is concluded that CpG sites get increasingly methylated as a function of age.

Earlier studies from this laboratory have shown that the chromatin of the brain gets increasingly condensed with age in rats as seen by its digestion by DNase I and nick-translation [24,25]. It has been reported that DNA methylation decreases in the brain (14%) and spleen (17%), but increases in the kidney (30%), and remains unaltered in the liver during aging of the rat [26]. These tissue-specific variations may represent the balance between DNA repair and cell replacement during aging. The loss of 5mC during aging is found largely in the moderately and highly RDS, and is believed to be the result of the failure to methylate fully patches of repaired DNA [27,28]. The rate and the extent of age-related decrease in 5mC content has also been correlated with the length of the life span of the organism [29].

Our studies show that several GAATTC and CCGG RDS exist in the genome of the rat brain. $\overset{m}{C}$pG doublets are present in these sequences, and are also randomly distributed throughout the genome. Though the overall 5mC content is reported to decrease with age, there are specific RDS flanked by CCGG which acquire more C5 methyls with increasing age. Both $\overset{m}{C}$pG and $\overset{m}{C}$pC doublets exist at CCGG sequences in the genome. Hemimethylated $\overset{m}{C}$pC sequences may serve as substrates for methyltransferase and acquire C5 methyls at the external C of the complementary strand. A 5 kb CCGG RDS containing $\overset{m}{C}$pG doublets has several internal GAATTC sequences. Thus there is an age-related increase in CH_3 groups at $\overset{m}{C}$pG and $\overset{m}{C}$pC doublets of CCGG sequences which are either organized into repetitive (satellite) DNA sequences of long and short periodicities or are randomly distributed throughout the genome of rat brain. Since repetitive DNA sequences have been implicated in brain-specific gene regulation [30], such changes in DNA methylation pattern may alter the conformation of the chromatin and influence the expression of genes in the brain during the aging of rat [31].

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