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Biochemical evidence of variability in the DNA repeat length in the chromatin of higher eukaryotes*

(chromatin structure/nucleosome/micrococcal nuclease)

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ABSTRACT **Biochemical evidence is presented which confirms that the DNA repeat length in micrococcal nuclease (spleen endonuclease, nucleate 3′-oligonucleotidohydrolase, EC 3.1.4.7) digests of Chinese hamster ovary chromatin is shorter than that of rat liver chromatin [J. L. Compton, R. Hancock, P. Oudet, and P. Chambon (1976) *Eur. J. Biochem.*, in press]. A survey of available cells has shown that the DNA repeat length of the chromatin of higher eukaryotes varies widely. A value of 196 base pairs was found for cells of all mature tissues, regardless of the source of the tissue, whereas smaller values were found for cells of actively dividing tissues and larger values were found for a genetically inactive cell. Although the DNA repeat length of the chromatin of cells in culture was usually shorter than 196 base pairs, there was no general correlation between the size of the chromatin DNA repeat length and the rate of cell division or the functional state of the cell in culture. Examination of extensive micrococcal nuclease digests suggests that the chromatin subunits of all of the higher eukaryotic cells we have studied contain a core with approximately 140 base pairs of DNA.**

Kornberg (1) proposed that the fundamental structure of chromatin is a flexibly jointed chain composed of a repeating subunit containing two each of histones H2A, H2B, H3, and H4, and 200 base pairs of DNA. In support of this model a subunit structure has been visualized by electron microscopy in interphase and mitotic chromatin (2–7). In addition, digestion of chromatin of higher eukaryotes with micrococcal (spleen endonuclease, nucleate 3′-oligonucleotidohydrolase, EC 3.1.4.7) nuclease produces DNA fragments which migrate on polyacrylamide gels as integral multiples of a repeat length of approximately 200 base pairs (refs. 8 and 9; for further references, see 10 and 11). In contrast, Lohr and Van Holde (12) reported that the chromatin DNA repeat length of yeast is approximately 135 base pairs in length which suggested that the subunit of chromatin may be different in higher and lower eukaryotes. Recently, Compton *et al.* (7) found that the repeat length of cell chromatin from Chinese hamster ovary (CHO) is 177 base pairs. This paper confirms this observation, excludes the possibility of artifacts arising from methods of nuclei preparation and nuclease digestion, and demonstrates that there are variations in the DNA length per chromatin subunit in higher eukaryotic cells.

MATERIAL AND METHODS

Sources of Cells. CHO, BHK (Syrian hamster kidney), CV1 (African green monkey), and HeLa cells are routinely cultured in this laboratory. CHO cells were also obtained from the laboratory of R. Hancock (Lausanne) (7). C6 cells (rat glial tumor cells) were provided by Y. Ciesielski (Strasbourg). P815 cells (mouse mastocytes) were provided by J. Gautschi (Bern). Teratoma, myoblast, and myotube cells (13) were provided by D. Paulin (Paris). Zajdela hepatoma cells, both as *in vivo* ascitic and cultured cells (14), and hepatoma HTC cells were provided by J. P. Beck (Strasbourg). Primary cultures (15) of rat kidney cells were prepared by J. E. Germond (Strasbourg). Micrococcal nuclease digests of *Physarum polycephalum* nuclei were provided by J. Stadler and R. Braun (Bern).

Preparation of Nuclei. Nuclei of cells in culture were prepared as follows (method I), with all steps performed at 0–4°. Cells were pelleted and resuspended in 10 mM Tris·HCl (pH 7.5), 1 mM $CaCl_2$ at a concentration of approximately 2×10^7 cells per ml. After 10 min, the cells were lysed in a Dounce homogenizer (B pestle). The lysate was layered over 3 ml of 1.7 M sucrose in 10 mM Tris·HCl (pH 7.5), 1 mM $CaCl_2$ and spun at $650 \times g$ for 1 hr. The nuclei were resuspended in the appropriate digestion buffer and used immediately. Nuclei of cells prepared from tissues were prepared according to Burgoyne *et al.* (16) (method II).

Micrococcal Nuclease Digestion. The CHO cells were digested with micrococcal nuclease (Worthington) in two ways. In method A, as previously described (7), the digestion buffer was 1 mM Tris·HCl (pH 8.0 at 37°), 1 mM $CaCl_2$ and the digestion was carried out at 37° until 2–4% of the DNA was rendered acid soluble. Digestion was terminated by addition of EDTA (5 mM final concentration). Method B was used for CHO and all of the other cells. Nuclei were resuspended at a DNA concentration of about 1 mg/ml in 15 mM Tris·HCl (pH 7.4), 60 mM KCl, 15 mM NaCl, 15 mM 2-mercaptoethanol, 0.15 mM spermine (Sigma), 0.5 mM spermidine (Sigma), 0.34 M sucrose, and 1 mM $CaCl_2$. Micrococcal nuclease was added, and digestion to determine the DNA repeat length was carried out at 20° until 2–4% of the DNA was rendered acid soluble, whereas the extensive digestion was done at 37° until 40–50% of the DNA was converted to acid soluble material. Digestion was terminated by the addition of EDTA (5 mM final concentration). Aliquots were acid precipitated to determine the amount of DNA which was rendered acid soluble. DNA was prepared by digestion for 1 hr at 37° with 50 μg of heat-treated pancreatic RNase (ribonuclease I, ribonucleate 3′-pyrimidino-oligonucleotidohydrolase, EC 3.1.4.22) (Sigma) and 2 μg of RNase T1 (guanyloribonuclease, ribonucleate 3′-guanylo-oligonucleotidohydrolase, EC 3.1.4.8) (Sigma) and overnight at 37° with proteinase K (Merck). The samples were phenol extracted and the DNA precipitated with ethanol as previously described (17).

Gel Electrophoresis. Polyacrylamide gels to determine the DNA repeat length were 2% acrylamide (19:1, acrylamide: bisacrylamide) and 0.5% agarose (Seakem), in running buffer, and were used in an 11-cm long slab apparatus at room tem-

Abbreviation: CHO, Chinese hamster ovary.

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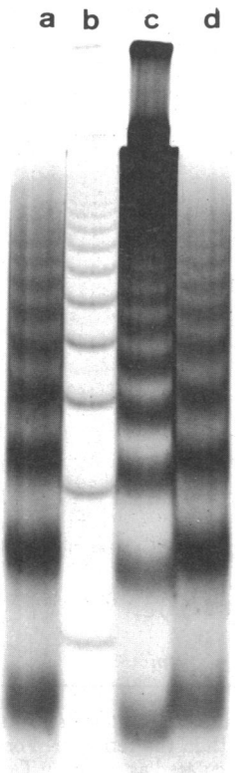

FIG. 1. Polyacrylamide gel electrophoresis of micrococcal nuclease digest of rat liver (slots a and d) and CHO cell (slot c) chromatins and *Eco*RII restriction fragments of mouse satellite DNA (slot b). Rat liver nuclei were prepared by method II and digested by method B. CHO cell nuclei were prepared by method I and digested by method A (see *Material and Methods*).

perature. The gel for the submonomer DNA fragments was 5.5% acrylamide/0.5% agarose and was used in a 17-cm long slab apparatus at room temperature. The running buffer was 40 mM Tris·HCl (pH 7.8), 20 mM sodium acetate, 2 mM EDTA. Approximately 1–5 μg of DNA was mixed with 10 μl of 40% sucrose (wt/vol) and was layered onto the gels under running buffer. The gels were run at 50 and 100 V for the short and long gels, respectively. Gels were stained in 0.5 μg/ml of ethidium bromide, transilluminated with short wavelength ultraviolet light, and photographed through a red filter. Gels were printed as negatives.

RESULTS

DNA Repeat Length Is Smaller in CHO Cell Chromatin Than in Rat Liver Chromatin. Fig. 1 shows a polyacrylamide gel with micrococcal nuclease digests of CHO and rat liver chromatin, and an *Eco*RII digest of mouse satellite DNA. Previous studies (17) have shown that the bands from an *Eco*RII digest of mouse satellite DNA migrate as integral multiples of a 245 base pair fragment when compared with restriction enzyme DNA fragments of known lengths. A calibration curve (not shown) for the gel in Fig. 1 was constructed by plotting the sizes of the DNA in the mouse satellite bands as a function of their relative mobilities (17). The sizes of the DNA from the CHO and rat liver digests were then determined from this calibration curve. The values obtained for the DNA bands are shown in Table 1. The length of the chromatin DNA repeat increases in the higher multiples until a plateau value is reached that begins with the pentamer. We attribute the smaller length of the DNA repeat in the lower multiples to exonucleolytic

Table 1. Length of DNA fragments shown in Fig. 1

Multiple*	Length of multiples (base pairs)	
	Rat liver	CHO cells
1	189 (189)	165 (165)
2	377 (189)	336 (168)
3	578 (193)	522 (174)
4	773 (193)	698 (175)
5	982 (196)	884 (177)
6	1175 (196)	1068 (178)
7	1378 (197)	1243 (178)
8	1568 (196)	1417 (177)
9	1767 (196)	1601 (178)
10	1969 (197)	1776 (178)

The DNA lengths of the multiples were determined from the relative mobilities of the centers of the bands (Fig. 1) as indicated in the *text*. The DNA repeat length (numbers in parentheses) was calculated by dividing the lengths of each multiple by the number of the band.

* The numbers correspond to the DNA bands shown in Fig. 1 beginning with the monomer.

digestion (9, 18–21). For this reason, all of the values which are presented in this paper correspond to plateau values which were determined from measurements of the migration of bands higher than the tetramer. In addition, because the measurements were made on enlargements taking the centers of the bands, early times in nuclease digestion were selected to avoid problems which could arise from the widening of the bands after more extensive digestion. As shown in Table 1, the lengths of the DNA repeat in rat liver and CHO chromatins were 196 and 178, respectively, in excellent agreement with previous observations (7, 17, 21). The same value for the DNA repeat length (178 base pairs) has been found for the chromatin of CHO cells from two independent sources. The rat liver digest shown in Fig. 1 was used throughout this study to calibrate the polyacrylamide gels.

Because both the method of nuclei preparation and digestion conditions were different for the CHO and rat liver digests shown in Fig. 1 (see *Material and Methods*), we have tested the effect of these variables on the size of the DNA subunit. Comparison of Fig. 2A and B shows that the size of the CHO DNA repeat length is the same irrespective of the method of digestion. A direct control of the nuclei preparation procedures proved to be impossible because the rat liver nuclei lysed spontaneously in the method I lysis buffer (i.e., in the absence of sucrose) and the CHO cells could not be lysed in the method II lysis buffer (see *Material and Methods*, and ref. 16). Consequently, CHO cells were lysed in the method I lysis buffer and the nuclei immediately pelleted and resuspended in the method II lysis buffer. All subsequent nuclei purification steps were performed according to method II (16). Fig. 2B and C show that there is no significant difference in the size of the DNA repeat length between CHO nuclei prepared by the two methods and digested according to method B. The difficulties in preparing nuclei described above proved to be general for other cells in culture and tissues. Consequently, nuclei from cells in culture were prepared by method I and nuclei from tissues were prepared by method II. All digestions to be described below were done as originally developed for the rat liver cells (method B).

DNA Repeat Lengths Smaller Than Those of Rat Liver Chromatin Are Not a General Characteristic of the Chromatin of Dividing Cells. To investigate whether variations in

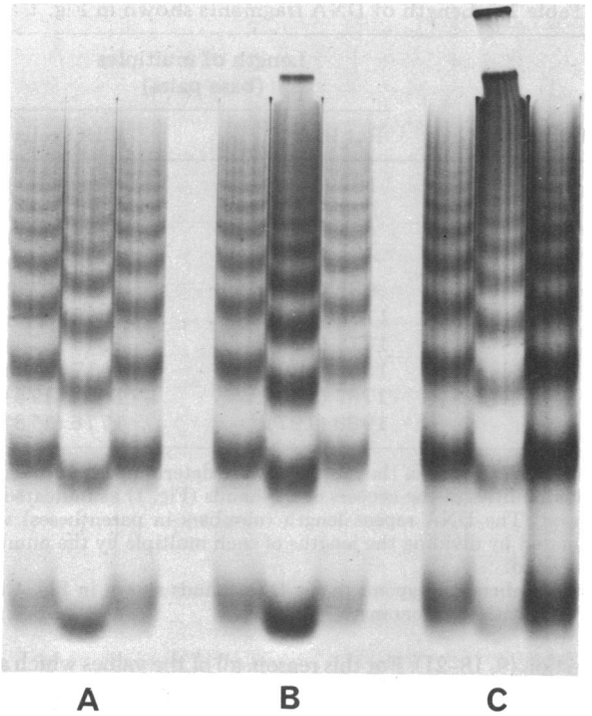

FIG. 2. Polyacrylamide gel electrophoresis of micrococcal nuclease digests of CHO cell chromatin prepared and digested in different ways. (A) Nuclei prepared by method I and digested according to method A; (B) nuclei prepared by method I and digested according to method B; (C) nuclei prepared by method II and digested according to method B. In each case the CHO chromatin digest is flanked by two rat liver digests.

the length of the DNA repeat could be found in the chromatin of other higher eukaryotes, we have screened a wide range of available cells in culture and tissues. Table 2 shows that there is no detectable difference between the DNA repeat lengths of chromatins of rat liver, rat kidney, Syrian hamster liver, Syrian hamster kidney, and chick oviduct. However, all but one of the actively dividing cells in culture that we have studied have a chromatin DNA repeat length smaller than that of the mature tissues. Values of 188–190 were found for HeLa, CV1, BHK, P815, ascitic, and cultured hepatoma, teratoma and myoblast cells. Polyacrylamide gels of DNA from micrococcal nuclease digests of chromatin of CV1 and ascitic hepatoma cells are shown in Fig. 3A and B. All of these cells have division times less than 24 hr. However, the chromatin of C6 cells, which also have a division time of less than 24 hr, has a DNA repeat length not significantly different from that of rat liver (Table 2 and Fig. 3C). Thus, a DNA repeat length shorter than that found in mature tissues does not appear to be a general characteristic of the chromatin of actively dividing cells. In support of this, we found that there are no differences in the DNA repeat lengths of chromatin of cells from exponentially growing and confluent cultures of CV1, cultured hepatoma and C6 cells (Table 2). In contrast, when cells from a mature tissue are induced to divide by putting them into primary cell culture, we observed a slight but significant decrease in the chromatin DNA repeat length (rat kidney cells, Table 2). It is also interesting that the DNA repeat length of the chromatin of actively dividing cells in two tissues, rat bone marrow and rat fetal liver, may be smaller than that of rat liver (Table 2).

Chromatin DNA Repeat Length in Relation to the Functional State of the Cell. We have attempted to relate the size of the DNA contained in the chromatin subunit to the functional state of the cell. No clear correlation is evident from our results. The teratoma-myoblast-myotube system offers a possibility of investigating two successive differentiation steps (13). As shown in Table 2, the chromatin DNA repeat length remains constant during the differentiation of teratoma cells to myoblast cells, whereas it increases when the myoblasts fuse to form myotubes. The hepatoma cell system offers the possibility of investigating the transition from ascitic hepatoma cells dividing *in vivo* and not synthesizing glycogen to *in vitro* cultured hepatoma cells which have regained the capacity to synthesize glycogen (14). As indicated in Table 2, there is no change in the chromatin DNA repeat length during this transition. Similarly, the erythrocyte of the chicken offers a possibility to examine the chromatin subunit in a genetically repressed, nondividing cell. Table 2 and Fig. 4A show that the DNA repeat contained in chicken erythrocyte chromatin is significantly larger than that of the chicken oviduct.

Table 2. DNA repeat length of various chromatins

Cell type	DNA repeat length* (base pairs)
Rat liver	196 ± 1
Rat kidney	196 ± 1 (3)†
Syrian hamster liver	196 ± 1 (2)
Syrian hamster kidney	196 ± 1 (2)
Chick oviduct	196 ± 1 (2)
CHO cells	178 ± 1 (7)
HeLa cells	188 ± 1 (3)
CV1 cells‡	189 ± 2 (6)
BHK cells	190 ± 2 (2)
P815 cells	188 ± 2 (2)
Hepatoma cells§	188 ± 2 (7)
Teratoma cells	188 ± 2 (6)
Myoblast cells	189 ± 2 (4)
Myotubes	193 ± 2 (3)
C6 cells‡	198 ± 2 (6)
Rat kidney primary culture	191 ± 2 (3)
Rat bone marrow	192 ± 1 (2)
Rat fetal liver (14 days)	193 ± 2 (2)
Chicken erythrocyte	207 ± 2 (3)
Physarum polycephalum	171 ± 2 (3)

* Determined as described in the *text* from the migration of center of the bands higher than the tetramer. For each band the DNA repeat length was calculated as shown in Table 1. The mean value of the DNA repeat lengths and its standard deviation were then calculated for each chromatin.

† Numbers of independent polyacrylamide gels that were measured.

‡ Exponentially growing or confluent cells.

§ The same value was obtained for HTC hepatoma cells and Zajdela hepatoma cells growing *in vivo* or *in vitro* [exponentially growing or confluent cells (32)].

Extensive Micrococcal Nuclease Digestions of All Examined Chromatins Produce a Common Pattern of DNA Fragments. Fig. 5 shows that in spite of variations in the DNA repeat length of chromatins of different cells, extensive digestion of these chromatins with micrococcal nuclease produces a common pattern of DNA fragments smaller than the monomer repeat length described above. These fragments have the same size as those which were previously described by several groups in extensive digests of cellular and simian virus 40 chromatins (7, 17, 19, 22, 23). Similar digestion patterns were obtained for all of the higher eukaryotic cells listed in Table 2 (not shown).

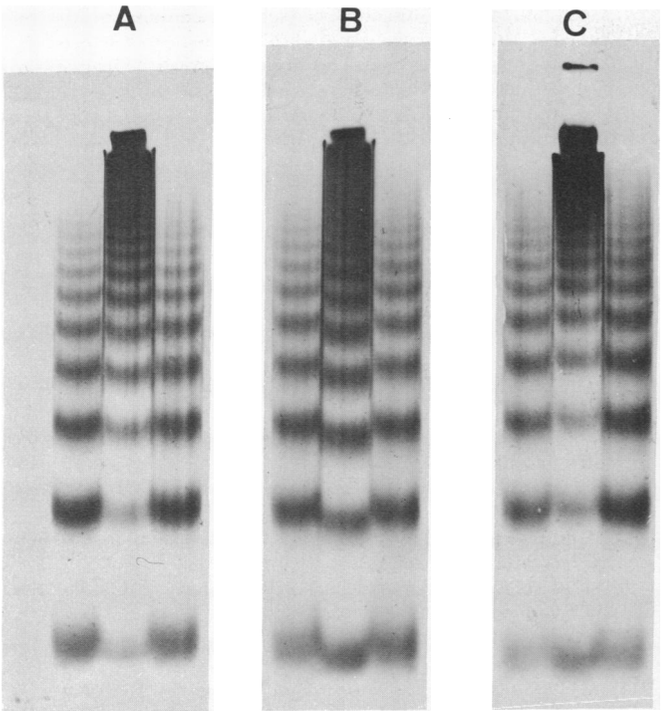

FIG. 3. Polyacrylamide gel electrophoresis of micrococcal nuclease digests of chromatins of (A) CV1 cells, (B) confluent Zajdela hepatoma cells grown *in vitro*, and (C) C6 cells. In each case the digest is flanked by two rat liver digests.

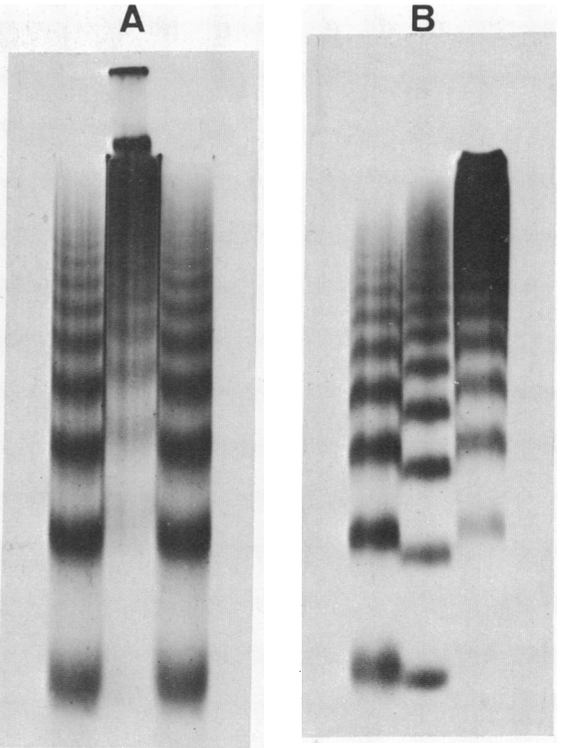

FIG. 4. Polyacrylamide gel electrophoresis of micrococcal nuclease digests of chromatins of (A) chicken erythrocyte and (B) *Physarum polycephalum*. In each case the digest is flanked by two rat liver digests.

DISCUSSION

In agreement with the model proposed by Kornberg (1), electron microscopic and biochemical studies have shown that the fundamental structure of chromatin of higher eukaryotic cells consists of a basic repeating subunit, the nucleosome, which contains two each of histones H2A, H2B, H3, and H4 and about 200 base pairs of DNA. Recent studies have shown that not all of the nucleosomal DNA is equally accessible to micrococcal nuclease (17, 19–23). This has led to the proposal that the nucleosome of higher eukaryotes contains a core consisting of two each of the four histones and approximately 140 base pairs of DNA which are more resistant to micrococcal nuclease digestion (19–23). In addition, evidence has been presented indicating the histone H1 interacts with at least some of the additional 60 base pairs of DNA which are associated with the nucleosome and which are more susceptible to the micrococcal nuclease digestion (21, 24). However, it has been recently shown that the DNA repeat length of chromatin of *Neurospora crassa* (25), and *Aspergillus nidulans* (26) cells is about 170 base pairs, although the DNA content of their nucleosome cores is also 140 base pairs. In addition, it appears that the DNA repeat length of yeast chromatin is also approximately 170 base pairs (25). Similarly, we have found that the DNA repeat length of another lower eukaryote, *Physarum polycephalum*, is about 170 base pairs (Fig. 4B and Table 2).

Our present results demonstrate that the length of DNA contained in the nucleosomes of higher eukaryotes is not invariable and ranges from 178 (CHO cells) to 207 base pairs (chicken erythrocytes). An even more extreme value for the DNA repeat length has recently been found for the chromatin of sea urchin sperm (241 base pairs, see ref. 27). However, our results suggest that all of the higher eukaryote chromatins that we have studied contain a similar nucleosome core organization. The results shown in Fig. 5 indicate that extensive micrococcal nuclease digestion results in the same pattern of sub-monomer DNA fragments irrespective of the origin of the chromatin. In addition, in all cases, the largest sub-monomer band is 140 base pairs in length. Therefore, the variability in the length of the DNA contained in the nucleosomes of different higher eukaryotic cells appears to arise from variability in the length of the extra-core DNA.

Noll (25) has suggested that the shorter DNA repeat length of *Neurospora crassa* chromatin when compared to that of higher eukaryotes could be related to a lower content of lysine residues in *Neurospora crassa* histone H1 which enables it to bind to a correspondingly shorter DNA length. This would result in a decrease of the DNA length between two nucleosome cores and consequently to a decrease of the DNA repeat length of the chromatin (25). A similar suggestion has been made by Morris (26) for *Aspergillus nidulans* chromatin. Similarly, it is possible that the longer DNA repeat length found in chicken erythrocyte chromatin is related to the replacement of histone H1 by the erythrocyte-specific histone H5. Whether variability of histone H1 (for references, see 28) could account for the observed variability of the chromatin DNA repeat length in eukaryotes is presently unknown and requires a detailed characterization of the H1 histones present in the various cells which we have studied. On the other hand the recent discovery of the existence of variants of histones H2A and H2B in animal cells (29, 30) raises the possibility that the variability in the chromatin DNA repeat could be related to modifications of the histone core.

Several lines of evidence indicate that the phosphorylation of histone H1, which is known to be associated with cell division (for references, see 28) and which is directly correlated to the rate of cell division (31), is not responsible for the observed variability of the chromatin DNA repeat length. Compton *et*

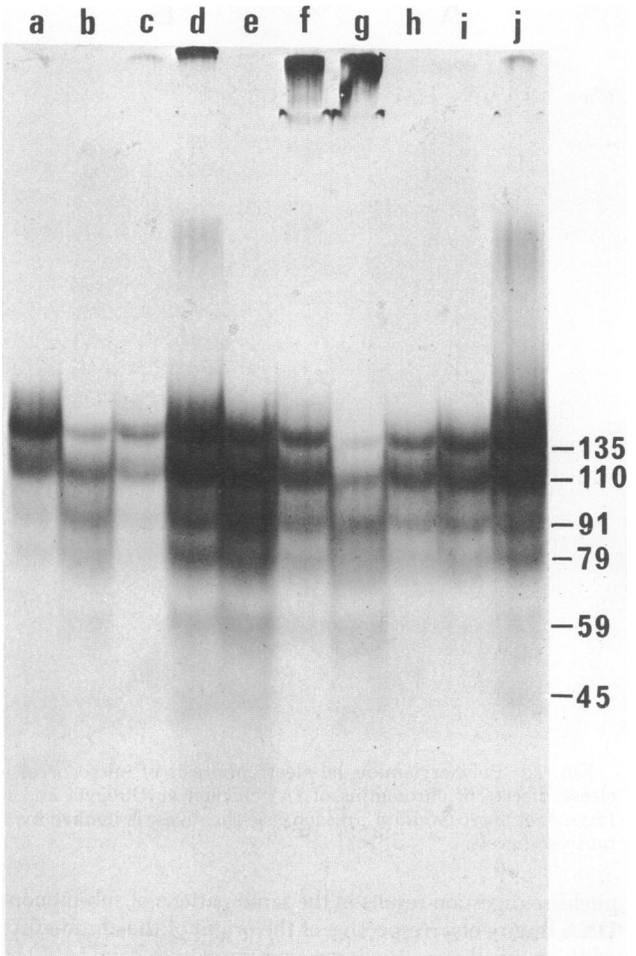

FIG. 5. Pattern of DNA fragments generated by extensive micrococcal nuclease digestion of various chromatins. After digestion (to 40–50% acid-soluble material) the deproteinized DNA was electrophoresed as described in *Material and Methods*. The gel was calibrated (in base pairs) as described (17). (a) Rat liver, (b) CHO cells, (c) CV1 cells, (d) HeLa cells, (e) C6 cells, (f) Zajdela hepatoma cells, (g) teratoma cells, (h) myoblast cells, (i) myotube cells, and (j) rat fetal liver.

al. (7) showed that the DNA repeat length of CHO chromatin is the same in interphase nuclei and mitotic chromosomes. We have shown that the chromatin DNA repeat length does not change when exponentially growing culture cells reach confluence. Furthermore, teratoma, CHO, and C6 cells which have approximately the same rate of cell division have different chromatin DNA repeat lengths. It thus appears that there is no general correlation between the size of the chromatin DNA repeat length and the stage in the cell cycle or the rate of cell division for cells in culture. In spite of some correlation between the length of the chromatin DNA repeat and the functional state of cells in living animals we must conclude at this time that the biological significance of the variability of the length of DNA in the chromatin subunit remains to be established.

We are greatly indebted to all of our colleagues who kindly provided us with cells and DNA. *Eco*RII mouse satellite digest was a gift from Dr. Hörz and Zachau (Munich). The technical assistance of Mrs. M. Acker and of Mr. B. Boulay is gratefully acknowledged. We thank Dr. P. Oudet for helpful discussion. This work was supported by grants of the Centre National de la Recherche Scientifique (ATP "Différenciation"—Contrat no. 2117; ATP "Internationale"—Contrat no. 2054), of the Institut National de la Sante et de la Recherche Medicale (Contrat no. 76.5.099.1) and of the Fondation pour la Recherche Médicale Française.

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