The Variation with Age of the Structure of Chromatin in Three Cell Types from Rat Liver

By VASSO ZONGZA and ANTHONY P. MATHIAS

Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

(Received 15 September 1978)

The organization of chromatin in three rat liver nuclear populations, namely diploid stromal, diploid parenchymal, and tetraploid parenchymal nuclei, which were separated by zonal centrifugation, was studied by digestion with micrococcal nuclease and pancreatic deoxyribonuclease in 3-week-old rats in which the parenchymal cells contain diploid nuclei and in 2- and 4-month-old rats with a high proportion of tetraploid nuclei. Digestion by micrococcal nuclease allowed the estimation of DNA-repeat length in chromatin. Parenchymal nuclei have shorter repeat length than stromal nuclei and DNA-repeat length increases with the age in all three nuclear populations. The kinetics of digestion by micrococcal nuclease showed that nuclei with short repeat length are more sensitive to micrococcal nuclease and that the sensitivity of chromatin decreases with age for all the types of nuclei in this study. The kinetics of digestion by pancreatic deoxyribonuclease showed that sensitivity of chromatin is related to the repeat length and that the sensitivity decreases with the age.

The chromatin of higher and lower eukaryotes consists of repeating nucleoprotein units, the so-called nucleosomes [for reviews see Kornberg (1977) and Felsenfeld (1978)]. Investigations with nucleases have revealed that each nucleosome consists of a resistant core particle of 140 base-pairs of DNA wrapped around an octamer of the four histones H2A, H2B, H3 and H4, and a DNA spacer, linking adjacent cores, and sensitive to nucleases (Sollner-Webb & Felsenfeld, 1975; Axel, 1975; Shaw *et al.*, 1976). The DNA-repeat length (estimated from mild digestion of chromatin) varies between different organisms, tissues of the same organism (Compton *et al.*, 1976; Morris, 1976a; Spadafora *et al.*, 1976; Morris, 1976b; Noll, 1976; Lipps & Morris, 1977; Lohr *et al.*, 1977a), cells of the same tissue (Thomas & Thompson, 1977), and even within a single cell type (Johnson *et al.*, 1976; Lohr *et al.*, 1977b; Martin *et al.*, 1977). DNA-repeat lengths so far reported vary from about 160 base-pairs for the fungi to 241 base-pairs for sea-urchin sperm. The length of 140 base-pairs of DNA in the core particle seems to be invariant in all chromatin samples examined so far, indicating that the variability is due to the variation of the length of linker DNA.

Many of the basic studies of chromatin structure have been performed on rat liver and its DNA-repeat length has been estimated as 198 ± 6 base-pairs (Noll & Kornberg, 1977). Liver, however, is an organ constituted of several classes of cells. These can be divided into two categories: parenchymal and stromal. Stromal cells include the bile duct cells, Kupfer cells, blood-vessel cells, connective tissue

cells and so forth. Therefore any sample of nuclei will be a complex mixture. A further complication arises from the polyploidy of parenchymal cells (for a review, see Carriere, 1969). The relative proportion of the diploid and tetraploid nuclei varies with the age of animals (Johnston *et al.*, 1968).

The present study concerns the organization of chromatin in three liver nuclear populations, namely the diploid stromal nuclei (2NS), the diploid parenchymal nuclei (2NP), and the tetraploid parenchymal nuclei (4NP). The accessibility of chromatin to two nucleases with a different mode of action, micrococcal nuclease and pancreatic DNAase (DNAase I), was examined and the nucleosomal DNA length was estimated by using the procedure of Noll & Kornberg (1977). Animals of three different ages were studied: 3-week-old rats, in which there are no polyploid nuclei and 2- and 4-month-old rats with a considerable proportion of tetraploid nuclei. The kinetics of chromatin digestion by both micrococcal nuclease and pancreatic DNAase (DNAase I) showed that parenchymal nuclei are more sensitive than stromal nuclei to nucleases and that generally accessibility decreases with the age. It is noteworthy that the DNA-repeat length of parenchymal nuclei is shorter than that of stromal nuclei and the DNA-repeat length increases with age for the three nuclear populations studied.

Experimental

Materials

Micrococcal nuclease (nucleate 3'-oligonucleotidohydrolase, EC 3.1.4.7) was purchased from P-L

Abbreviation used: DNAase, deoxyribonuclease.

Biochemicals Inc., Milwaukee, WI 53205, U.S.A. DNAase I (deoxyribonuclease 5'-oligonucleotidohydrolase, EC 3.1.4.5; grade I), and proteinase K were purchased from Boehringer Corp. (London), Ealing, London W5 2TZ, U.K. Spermine and spermidine were purchased from Sigma (London) Chemical Co., Kingston upon Thames, Surrey KT2 7BH, U.K. Restriction fragments of bacteriophage Φ X174 RF DNA with *Hae* III restriction endonuclease were purchased from Bethesda Research Laboratories Inc., Rockville, MD 20850, U.S.A.

Preparation of nuclei

Male Sprague-Dawley rats were fed on a GR3 E.K. diet, and had access to the food and water *ad libitum*. They were starved overnight before being killed. Livers were removed from 3-week-, 2-month- and 4-month-old rats that had been killed by cervical dislocation. Minced tissue was homogenized in 7 vol. of 0.34 M-sucrose/3 mM-MgCl₂, adjusted to pH 7.4 with a saturated solution of NaHCO₃, in a Potter-Elvehjem homogenizer with a motor-driven Teflon pestle (clearance 0.25 mm) with 15 strokes at 1700 rev./min, filtered through nylon bolting cloth and centrifuged at 1000g for 10 min. The nuclear pellet was resuspended in 6 vol. of 2.1 M-sucrose/1 mM-MgCl₂/NaHCO₃, pH 7.4, layered over an equal volume of the same solution and centrifuged at 70000g for 1 h. The nuclear pellet was resuspended in 0.37 M-sucrose/1 mM-MgCl₂/NaHCO₃, pH 7.4, and nuclei were separated in different classes by zonal centrifugation as described by Johnston *et al.* (1968). Nuclei were recovered from the effluent, examined by phase-contrast microscopy and counted by using a Coulter counter as described by Johnston *et al.* (1968). Nuclei that were to be digested with micrococcal nuclease were washed with buffer A [15 mM-Tris/HCl (pH 7.4), 60 mM-KCl, 15 mM-NaCl, 15 mM-2-mercaptoethanol, 0.15 mM-spermine, 0.5 mM-spermidine]/0.34 M-sucrose/2 mM-EDTA/0.2 mM-EGTA (Hewish & Burgoyne, 1973), and finally suspended in buffer A/0.34 M-sucrose. Nuclei that were to be digested with DNAase I were washed in 0.34 M-sucrose/1 mM-MgCl₂/NaHCO₃, pH 7.4, and finally suspended in the same buffer. Portions were kept for the analysis of DNA (Burton, 1956) and the rest of the nuclear suspensions were immediately frozen in liquid N₂ and stored at -70°C. No changes were observed in nuclease-digestion patterns during 2 weeks of storage under these conditions.

Nuclease digestions

Micrococcal nuclease digestion. Nuclear samples were adjusted to a concentration of 1 mg of nuclear DNA/ml and 10 mM-CaCl₂ was added to a final concentration of 1 mM. The mixture was preincubated

for 2 min at 37°C. Nuclei were digested with micrococcal nuclease (375 units/mg of DNA) at 37°C for various times. One unit of micrococcal nuclease produces 1.00 A_{280} unit of acid-soluble material with 2.5 mg of DNA/ml as substrate at pH 8.8 and 37°C. The reaction was terminated by the addition of 100 mM-EDTA to a final concentration of 5 mM and by chilling on ice. To determine the amount of DNA that was rendered acid-soluble, portions were precipitated with an equal volume of 1 M-HClO₄ and centrifuged for 4 min in an Eppendorf centrifuge. The A_{260} of the supernatant was measured and divided by 1.68 to correct for the hyperchromic effect of micrococcal digestion, as described by Noll & Kornberg (1977). Control samples were similarly treated except that no enzyme was added.

DNAase I digestions. Nuclei were suspended in 0.34 M-sucrose/1 mM-MgCl₂/NaHCO₃, pH 7.4, at a concentration of 0.4–0.5 mg of DNA/ml. MgCl₂ (50 mM) was added to a final concentration of 2 mM and the mixture was preincubated for 2 min at 37°C. The reaction was started by the addition of 40 units of DNAase I/mg of DNA. One unit of DNAase I activity causes an increase in A_{260} of 0.001/min per ml at 25°C and with DNA as substrate. Portions were removed at various times and added to an equal volume of 1 M-HClO₄ for the determination of acid-soluble material. Control samples were incubated for the same periods of time, but in the absence of enzyme, and the acid-soluble material was determined.

Extraction of DNA

Samples were made 0.5% in sodium dodecyl sulphate. Proteinase K was added to give a concentration of 200 μ g/ml, and the mixtures were incubated for 2 h at 37°C. The concentration of sodium dodecyl sulphate was increased to 1% and NaCl was added to 1 M. The samples were extracted twice with chloroform/isopentyl alcohol (24:1, v/v). DNA was precipitated with 2 vol. of ethanol for at least 3 h at -40°C. The pellets were dried under N₂ and dissolved in electrophoresis buffer diluted 100-fold (see below) to give an A_{260} value of about 38.

Gel electrophoresis

Gel electrophoresis of DNA samples was carried out in vertical 2.5% acrylamide (acrylamide/bisacrylamide, 19:1)/0.5% agarose slab gels (8 cm long) with the Tris/borate buffer system [0.9 M-Tris/HCl (pH 8.3), 25 mM-EDTA, 0.88 M-boric acid] of Peacock & Dingman (1968). The gel for the submononucleosome DNA fragments was 6% acrylamide (acrylamide/bisacrylamide, 29:1) with the same buffer system.

To 50 μ l samples of the solutions of DNA, 10 μ l of 60% (w/v) sucrose and 0.1% (w/v) Bromophenol

Blue was added and 2 μ l was applied to each slot. Gels were run at 75 V for about 1 h 30 min or until the Bromophenol Blue marker was 1 cm above the bottom of the gel. Gels were stained in a solution of 3 μ g of ethidium bromide/ml for 30 min. DNA was visualized in the gels under u.v. light and photographed through an orange filter. The negatives were scanned with a two-dimensional scanner, designed and made by Dr. D. Ridge, Department of Biochemistry, University College London. The peak areas were determined by the weight method.

Results

Digestion with micrococcal nuclease and estimation of the DNA-repeat length

For the measurement of the DNA-repeat length, nuclei were digested with micrococcal nuclease, as described in the Experimental section, to the same extent, usually to the point at which 4% of the original DNA had become acid-soluble. To ensure strict comparability it is essential to examine on gels samples digested to the same degree because the amount of material in a band may affect the migration of the neighbouring bands and lengthening the time of digestion may shorten the DNA fragments.

The band sizes were obtained from a calibration graph (Fig. 2) constructed with the use of restriction fragments of bacteriophage Φ X174 RF DNA run in the same slab gel (Fig. 1). The values for the DNA-repeat lengths in the various nuclei were measured by taking the slope of a graph of band size (in base-pairs) against band number (Fig. 3), as in the method of Noll & Kornberg (1977). In this way the effect of over-digestion is eliminated. Table 1 gives the band sizes of multiples up to a unit size of seven, for the samples shown in Fig. 1. As Table 2 shows, the nucleosomal DNA length varies in the three types of nuclei and in the three ages. Parenchymal nuclei appear to have shorter DNA-repeat length than stromal nuclei in all the ages studied. Diploid parenchymal nuclei have a slightly longer DNA-repeat length than tetraploid parenchymal nuclei in the 2-month-old rats and the reverse occurs in 4-month-old rats. However, the difference is 3 base-pairs only and probably is not significant. Parenchymal nuclei are known to be more active in transcription than stromal nuclei (Gonzalez-Mujica & Mathias, 1973a). It is also known from the same study that in the case of 2-month-old rats, 4NP nuclei show a higher RNA polymerase activity than 2NP nuclei. So the short DNA-repeat length could be a consequence of the packaging of chromatin in 'active' nuclei, a consideration that has been suggested previously (Morris, 1976b; Thomas & Thompson, 1977). On the other hand, the length of DNA-repeat unit increases with the age and differences between

Fig. 1. A photograph of a typical gel for the determination of the size of DNA fragments obtained from digestion by micrococcal nuclease of diploid stromal (2NS), diploid parenchymal (2NP), and tetraploid parenchymal (4NP) liver nuclei

Nuclei were digested until 4% of the original DNA was rendered acid-soluble. DNA was purified as described in the Experimental section and electrophoresed in 2.5% acrylamide (acrylamide/bisacrylamide, 19:1)/0.5% agarose slab gels (8 cm long) with the Tris/borate system of Peacock & Dingman (1968). Electrophoresis was carried out at room temperature (18°C) at 75 V for 1 h 30 min. Abbreviation: *Hae*, *Hae* III restriction endonuclease digest of bacteriophage Φ X174 RF DNA.

Fig. 2. A typical calibration curve for determining the size of DNA fragments in polyacrylamide gels

Distances of migration were measured from the origin to the midpoint of the bands in the photograph of the gel shown in Fig. 1. ○, *Hae* III restriction endonuclease digest of bacteriophage Φ X174 RF DNA.

Fig. 3. Two typical graphs for the determination of DNA-repeat lengths in the liver nuclear populations studied. The sizes of DNA bands from 2NS, 2NP and 4NP nuclei in the three ages shown in Fig. 1 were estimated from the calibration curve of Fig. 2. The distances of migration were measured to the midpoints of the bands on the gels. The DNA-repeat length is determined from the slope of the graph. 2NP nuclei from 3-week-old rats ($\square$, upper abscissa scale) and 4-month-old rats ($\circ$, lower-abscissa scale).

the three types of liver nuclei are minimized in the older (4-month-old) rats. Digestion of unfractionated nuclear liver preparations give DNA-repeat lengths that are intermediate between those of individual nuclear populations. The DNA length in core particles for the fractionated nuclei, estimated from electrophoresis of samples digested to the extent of 20% acid solubility (as described in the Experimental section), was found to be 135–140 base-pairs for the three types of nuclei.

Kinetics of digestion by micrococcal nuclease of the three nuclear classes of rat liver

The kinetics of digestion of chromatin with micrococcal nuclease, based on the determination of the amount of DNA that is rendered acid-soluble (Fig. 4), has shown that 2NP nuclei, compared with 2NS nuclei, are more easily attacked by micrococcal nuclease in 3-week- and 2-month-old rats. Tetraploid nuclei in 2-month-old rats appear to have the same or slightly less sensitivity to micrococcal nuclease, compared with diploid parenchymal nuclei. It seems that a difference of three base-pairs in the DNA-repeat length between 2NP and 4NP nuclei is insignificant and no real difference in accessibility to micrococcal nuclease can be detected. In 4-month-old rats where the DNA-repeat lengths of all three types of nuclei are very close to each other, little difference

Table 1. Sizes (in base-pairs) of DNA fragments produced by micrococcal nuclease digestion of rat liver nuclear classes. The sizes of the DNA fragments in the polyacrylamide gels, one of which is shown in Fig. 1, were estimated from the calibration curve shown in Fig. 2. Mobilities were measured from the origin to the midpoints of the bands on gel photographs.

		Size of DNA fragment (number of base-pairs)								
		3 weeks			2 months			4 months		
Oligomer size (no. of units)	Type of nucleus	2NS	2NP	4NP	2NS	2NP	4NP	2NS	2NP	4NP
1		164	170		171	171	167	170	170	170
2		378	366		385	385	385	377	358	370
3		582	583		609	609	582	599	579	590
4		776	782		826	820	800	806	795	821
5		961	939		1000	994	961	1018	983	1001
6		1170	1140		1225	1196	1160	1225	1205	1224
7		1360	1335		1450	1389	1375			

Table 2. DNA-repeat lengths of the three nuclear liver populations at three ages. Repeat lengths were determined by linear regression as described in the text and in Fig. 3.

		Average repeat length measured by linear regression (base-pairs)		
Age	Type of nuclease	Diploid stromal (2NS)	Diploid parenchymal (2NP)	Tetraploid parenchymal (4NP)
3 weeks		198 $\pm$ 1	193 $\pm$ 3	—
2 months		211 $\pm$ 3	202 $\pm$ 3	199 $\pm$ 3
4 months		211 $\pm$ 1	208 $\pm$ 2	211 $\pm$ 3

Fig. 4. Time course of digestion of liver nuclei by micrococcal nuclease

Nuclei were digested with micrococcal nuclease for various times, and the amount of DNA that was rendered acid-soluble was determined as described in the Experimental section. The values shown are the means for duplicates with the control values subtracted. (a) 3-week-old rats; (b) 2-month-old rats; (c) 4-month-old rats. Symbols: $\square$, 2NS nuclei; $\circ$, 2NP nuclei; Δ , 4NP nuclei.

between the nuclear types can be detected. It is also noteworthy that, whereas the sensitivity of chromatin to micrococcal nuclease decreases with age, the DNA-repeat length increases with age. These experiments have been repeated four times and show good reproducibility.

The accessibility of chromatin to micrococcal nuclease was also studied by quantification of monomer and oligomer bands that appear on the gel. DNA from nuclei digested for various times was purified and run on gels as described in the Experimental section. The negatives of the photographs were scanned and the amount of DNA that appears in the form of mononucleosome was measured by the weight method. Fig. 5 gives one typical scan and Fig. 6 gives the graphs of the percentage of mononucleosome against time for the three nuclear populations in the three ages of animals used. The amount of mononucleosome was calculated as a percentage of the total amount that appears in bands on the gel. Although the same amounts of DNA, as determined by absorption, were applied on the gels for all the samples, the amount of DNA on the stained gel decreases with the time of digestion, as acid-soluble material and submononucleosomal material increases. Mononucleosomes are also readily broken down by micrococcal nuclease to form a series of submononucleosomal DNA fragments (Fig. 7). The general conclusion can be drawn from Figs. 6 and 7 that nuclei with short repeat lengths and high amounts of acid-soluble DNA have less DNA in the form of mononucleosomes, as mononucleosomes themselves are further digested to smaller DNA fragments. A possible explanation is discussed below.

Fig. 5. A typical densitometric scan of the negative of a gel photograph with the micrococcal nuclease-digestion products

Nuclei were incubated for various times in the presence of micrococcal nuclease. DNA was purified and electrophoresed as described in the Experimental section. Gels were photographed under u.v. light and the negatives were scanned. Diploid parenchymal nuclei from 3-week-old rats were used. (a) Control, incubated for 8 min at 37°C without enzyme; (b) 30s digest; (c) 2min digest; (d) 4min digest; (e) 8min digest.

Fig. 6. Time course of mononucleosome production during micrococcal nuclease digestion

Densitometric scans of the negatives of gel photographs shown in Fig. 5 were quantified and the amount of mononucleosome was calculated as a percentage of the total DNA that appears in bands on the gel. (a) 3-week-old rats; (b) 2-month-old rats; (c) 4-month-old rats. Symbols: $\square$, 2NS nuclei; $\circ$, 2NP nuclei; Δ , 4NP nuclei.

Kinetics of digestion by DNAase I of the three liver nuclear populations

Nuclei were digested with DNAase I for various times and the amount of DNA that was rendered acid-soluble was determined. As Fig. 8 shows, diploid parenchymal nuclei with short repeat lengths are more accessible to DNAase I than stromal diploid nuclei. Tetraploid nuclei at the age of 2 months are more resistant to DNAase I than diploid parenchymal nuclei, although the repeat length of the former is shorter than that of the latter. At the age of 4 months the accessibility of 2NP and 4NP nuclei to DNAase I is about the same, with the 2NP nuclei being slightly more accessible. In general, however, only small differences in accessibility of various kinds of nuclei that we studied to DNAase

Fig. 7. A photograph of a typical gel of the digestion products of micrococcal nuclease

Nuclei were digested for various times by micrococcal nuclease. DNA was purified and electrophoresed as described in the Experimental section except that electrophoresis was carried out for 1 h to reveal the subnucleosomal material that otherwise runs out of the gel. Diploid parenchymal nuclei from 2-month-old rats were used. (a) 8 min digest; (b) 4 min digest; (c) 2 min digest; (d) 30 s digest.

I are observed. Again the sensitivity of chromatin in the three liver nuclear populations to DNAase I decreases with age, which also occurs with micrococcal nuclease.

Discussion

The present results show that the three classes of liver nuclei differ in the packaging of their chromatin and also that the packaging of chromatin changes with age. As liver is commonly used in many biochemical studies this is very important. The difference in DNA-repeat length between parenchymal and stromal nuclei is 5 base-pairs in 3-week-old rats, increases to about 10 base-pairs in 2-month-old rats and almost vanishes in 4-month-old rats. In 2-month-old rats 4NP nuclei have a shorter repeat length than 2NP nuclei. It has been shown by Gonzalez-Mujica & Mathias (1973a) that the RNA polymerase activity is higher in parenchymal nuclei compared with stromal nuclei, and in 2-month-old rats 4NP nuclei have higher RNA polymerase activity than 2NP nuclei. Thus it seems that the difference in repeat length may have a functional significance.

A correlation of high transcriptional activity and short repeat length and vice versa agrees probably with the occurrence of short repeat lengths in

Fig. 8. Time course of digestion of liver nuclei by DNAase I

Nuclei were digested with DNAase I and the amount of DNA that was rendered acid-soluble was determined as described in the Experimental section. (a) 3-week-old rats; (b) 2-month-old rats; (c) 4-month-old rats. Symbols: $\square$, 2NS nuclei; $\circ$, 2NP nuclei; $\triangle$, 4NP nuclei.

actively growing cells in *Paramecium aurelia* [153 ± 7 (mean $\pm$ s.d.) base-pairs] and *Tetrahymena pyriformis* [207 ± 10 (mean $\pm$ s.d.) base-pairs] compared with stationary growing cells [178 ± 6 and 230 ± 10 (mean $\pm$ s.d.) base-pairs respectively] (Prince *et al.*, 1977), in chick erythroblasts [190 ± 3 (mean $\pm$ s.d.) base-pairs] compared with erythrocytes (212 base-pairs) (Weintraub, 1978), in sea-urchin gastrula (218 base-pairs) compared with transcriptionally inert sea-urchin sperm (241 base-pairs) (Spadafora *et al.*, 1976), and in neuronal nuclei of cerebral cortex [162 ± 6 (mean $\pm$ s.d.) base-pairs] compared with non-astrocytic glial-cell nuclei [197 ± 6 (mean $\pm$ s.d.) base-pairs] (Thomas & Thompson, 1977). It could be argued that only the small proportion of the genome that is actively engaged in transcriptional processes would change its packaging. It is known that DNA-repeat length varies within a single cell type (Johnson *et al.*, 1976; Lohr *et al.*, 1977b; Martin *et al.*, 1977). The DNA-repeat length is measured in the overall products of the digestion and consequently the DNA-repeat length obtained is a mean value for the repeat lengths in the genome. Possibly, therefore, the changes in the active regions of the genome may be even more dramatic than is suggested by the overall values.

The kinetics of digestion by DNAase I show that parenchymal nuclei are slightly more sensitive to DNAase I than stromal nuclei. This correlates with the shorter repeat lengths found for parenchymal nuclei and their higher RNA polymerase activity compared with stromal nuclei. It is known that DNAase I preferentially digests sequences active in transcription (Weintraub & Groudine, 1976; Garel & Axel, 1977; Flint & Weintraub, 1977), and it has been suggested by Weintraub & Groudine (1976)

that the nucleosome structure in 'active sequences' is modified or opened in a way that permits pancreatic DNAase to detect the difference. However, there is no clear evidence of a specific gene digestion in the present study and the differences in sensitivity observed are rather small.

The kinetics of digestion by micrococcal nuclease are more or less similar to those by DNAase I, but greater differences in sensitivity are found. The kinetics of production of the mononucleosome (expressed as a percentage of the total DNA that appears in bands on the stained gel) show that in nuclei with a short repeat length and DNA that is relatively easily released as acid-soluble material the percentage of mononucleosomes that is released is low. This could be explained if we assume that in chromatin with short repeat lengths the nucleosome structure is opened up in a way that is sensitive to micrococcal nuclease, and the mononucleosomes, as they are liberated, are digested to submononucleosomal particles and acid-soluble material, an assumption that is clearly supported by the experiment shown in Fig. 7. Reports in the literature on the mode of action of micrococcal nuclease are contradictory. Some of them (Lacy & Axel, 1975; Weintraub & Groudine, 1976) suggest that micrococcal nuclease does not discriminate between active and inactive sequences, whereas others suggest that it does (Bloom & Anderson, 1978; Bellard *et al.*, 1979). The present experiments are not conclusive on this point.

There are many possible explanations for the variation of the packaging of the genome in the different types of nuclei.

The fact that the DNA-repeat length varies not only between different types of cells in the same tissue, but, and this is the crucial point, changes with

age, invites speculation. It is possible, albeit unlikely, that as animals grow old there is a small increase in the amount of DNA per cell unaccompanied by an increase in the amount of histones. More probably the diploid amount of DNA per cell remains constant. If this is so, then the change in the DNA-repeat length could represent a minor decrease in the ratio of total histones to DNA, or the ratio of histones to DNA could remain constant. This would require that there was some age-dependent modification to the histones so that the histone core was able to interact only with a shorter length of DNA.

The overall charge of the individual histones is very important since histones with lower positive charge could be the cause of a shorter repeat length. Consequently histone modifications, such as acetylation and phosphorylation (Wallace *et al.*, 1977), could play a significant role in determining the packaging of chromatin and might regulate its biochemical activity (Letnansky, 1978). Histone H1 is probably the most important histone in this respect, since it is bound to the linker DNA (Whitlock & Simpson, 1976; Noll & Kornberg, 1977). As Weintraub (1978) has shown, the repeat length in erythrocytes increases when histone H1 is replaced by the more basic histone H5. Concentrations of histone H1 increase with the age in rat liver (Medvedev *et al.*, 1977), and this is a possible explanation for the increase in the DNA-repeat length with age that we have found in the three liver nuclear populations.

An alternative explanation would focus on the role of non-histone chromosomal proteins. One or more non-histone proteins could change their interaction with histones to achieve the increase in the DNA-repeat length. The role of non-histone chromosomal proteins in chromatin organization is not very clear at the moment. Two recent reports (Adolph *et al.*, 1977a,b), however, show clearly the significance of non-histone proteins in the structure of chromosomes. As Gonzalez-Mujica & Mathias (1973b) have shown, non-histones vary in the three liver classes of nuclei. We believe that the study of histone H1 and non-histone proteins at the level of the mononucleosome and dinucleosome is required if we are to understand the organization of chromatin in the nuclei we have used.

V. Z. was supported by a NATO fellowship and is grateful to her husband Dr. G. Dimitriadis for his moral support and many critical discussions during this work. We thank Dr. D. Ridge for help during zonal runs and Mr. D. Cox for excellent technical assistance.

References

- Adolph, K. W., Cheng, S. M. & Laemmli, U. K. (1977a) *Cell* **12**, 805–816
- Adolph, K. W., Cheng, S. M., Paulson, J. R. & Laemmli, U. K. (1977b) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4937–4941
- Axel, R. (1975) *Biochemistry* **14**, 2921–2925
- Bellard, M., Cannon, F. & Chambon P. (1979) *Cold Spring Harbor. Symp. Quant. Biol.* in the press
- Bloom, K. S. & Anderson, J. N. (1978) *Cell* **15**, 141–150
- Burton, K. (1956) *Biochem. J.* **62**, 315–323
- Carriere, R. (1969) *Int. Rev. Cytol.* **25**, 201–277
- Compton, J. L., Bellard, M. & Chambon, P. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4382–4386
- Felsenfeld, G. (1978) *Nature (London)* **271**, 115–122
- Flint, S. J. & Weintraub, H. M. (1977) *Cell* **11**, 933–940
- Garel, A. & Axel, R. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3966–3970
- Gonzalez-Mujica, F. & Mathias, A. P. (1973a) *Biochem. J.* **132**, 163–183
- Gonzalez-Mujica, F. & Mathias, A. P. (1973b) *Biochem. J.* **133**, 441–455
- Hewish, D. R. & Burgoyne, L. A. (1973) *Biochem. Biophys. Res. Commun.* **52**, 504–510
- Johnson, E. M., Littau, V. C., Allfrey, V. G., Bradbury, E. M. & Matthews, H. R. (1976) *Nucleic Acid Res.* **3**, 3313–3329
- Johnston, I. R., Mathias, A. P., Pennington, F. & Ridge, D. (1968) *Biochem. J.* **109**, 127–135
- Kornberg, R. D. (1977) *Annu. Rev. Biochem.* **46**, 931–954
- Lacy, E. & Axel, R. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3978–3982
- Letnansky, K. (1978) *FEBS Lett.* **89**, 93–97
- Lipps, H. J. & Morris, N. R. (1977) *Biochem. Biophys. Res. Commun.* **74**, 230–234
- Lohr, D., Gorden, J., Tatchell, K., Kovacic, R. T. & Van Holde, K. E. (1977a) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 79–83
- Lohr, D., Kovacic, R. T. & Van Holde, K. E. (1977b) *Biochemistry* **16**, 463–471
- Martin, D. Z., Todd, R. D., Lang, D., Pei, P. N. & Garrard, W. T. (1977) *J. Biol. Chem.* **252**, 8269–8277
- Medvedev, Z. A., Medvedeva, M. N. & Huschtscha, L. I. (1977) *Gerontology* **23**, 334–341
- Morris, N. R. (1976a) *Cell* **8**, 357–364
- Morris, N. R. (1976b) *Cell* **9**, 627–632
- Noll, M. (1976) *Cell* **8**, 349–355
- Noll, M. & Kornberg, R. D. (1977) *J. Mol. Biol.* **109**, 393–404
- Peacock, A. C. & Dingman, W. C. (1968) *Biochemistry* **7**, 668–674
- Prince, D. J., Cummings, D. I. & Seale, R. L. (1977) *Biochem. Biophys. Res. Commun.* **79**, 190–197
- Shaw, B., Herman, T., Kovacic, R. T., Beaudreau, G. & Van Holde, K. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 505–509
- Sollner-Webb, B. & Felsenfeld, G. (1975) *Biochemistry* **14**, 2915–2920
- Spadafora, C., Bellard, M., Compton, J. L. & Chambon, P. (1976) *FEBS Lett.* **69**, 281–285
- Thomas, J. O. & Thompson, R. J. (1977) *Cell* **10**, 633–640
- Wallace, B. R., Sargent, T. D., Murphy, R. F. & Bonner, J. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3244–3248
- Weintraub, H. (1978) *Nucleic Acid Res.* **5**, 1179–1188
- Weintraub, H. & Groudine, M. (1976) *Science* **193**, 848–856
- Whitlock, J. P., Jr. & Simpson, R. T. (1976) *Biochemistry* **15**, 3307–3314