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Organization of 5S genes in chromatin of Xenopus laevis

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ABSTRACT

The chromatin organization of the genes coding for 5S RNA in Xenopus laevis has been investigated with restriction endonucleases and micrococcal nuclease. Digestion of nuclei from liver, kidney, blood and kidney cells maintained in culture with micrococcal nuclease reveals that these Xenopus cells and tissues have shorter nucleosome repeat lengths than the corresponding cells and tissues from other higher organisms. 5S genes are organized in nucleosomes with repeat lengths similar to those of the bulk chromatin in liver (178 bp) and cultured cells (165 bp); however, 5S gene chromatin in blood cells has a shorter nucleosome repeat (176 bp) than the bulk of the genome in these cells (184 bp). From an analysis of the 5S DNA fragments produced by extensive restriction endonuclease cleavage of chromatin in situ, no special arrangement of the nucleosomes with respect to the sequence of 5S DNA can be detected. The relative abundance of 5S gene multimers follows a Kuhn distribution, with about 57% of all HindIII sites cleaved. This suggests that HindIII sites can be cleaved both in the nucleosome core and linker regions.

INTRODUCTION

It is now well established that eukaryotic chromatin is organized into repeating subunits or nucleosomes composed of about 200 base pairs (bp) of DNA in association with two molecules each of the histones H2A, H2B, H3 and H4 (the histone octamer) and one molecule of histone H1 (for review see ref. 1). The length of nucleosome-associated DNA is variable; differences between organisms and even between cell types of the same organism have been found (1). However, a common nuclease resistant core of 146 bp of DNA in association with the histone octamer has been found in all organisms examined. Histone H1 is thought to lie in the variable-length linker region (2) and differences in H1 histones are thought to be responsible for DNA length variation in this region of the chromatin fiber (3-4). Analysis of the reassociation kinetics of monomer nucleosome DNA suggest that most, if not all, nuclear DNA sequences are represented in the subunit structure (5). No

frequency class of DNA is absent from nucleosomes: highly repetitive sequences such as satellite DNAs (6-9) are organized in nucleosomes as are both moderately repetitive and single copy sequences (5). DNA sequences which are transcribed into messenger RNA are also represented in nucleosomal DNA (5). It is now apparent, however, that nucleosomes of active gene chromatin are structurally different from the nucleosomes of inactive genes. Active genes are highly sensitive to nucleolytic attack: DNaseI preferentially destroys active genes in chromatin (10-11), while DNaseII and micrococcal nuclease preferentially cleave active gene chromatin (12-14).

This paper reports initial investigations on the structure of the 5S RNA genes in chromatin of the frog Xenopus laevis. The DNA sequences coding for 5S RNA form a complex multigene family. 5S genes are present in many thousand copies per genome and different types of 5S genes are expressed in oocytes and somatic cells. Of the 5S genes, the major oocyte-type sequence is present in about 15,000 copies per haploid genome, thus comprising 0.38% of Xenopus DNA (15), and is organized in clusters of tandem repeating units. This gene has been sequenced (16-17): each 5S DNA repeat consists of about 700 bp, comprising an A+T rich spacer, the gene, G+C rich spacers and a pseudogene sequence. No stable transcripts of the pseudogene sequence have been detected. Both sequence and length heterogeneity have been noted in the major oocyte-type 5S DNA (16,18).

Reeves and Jones (19) and Humphries et al. (18) have shown that 5S genes are organized in nucleosomes. This paper extends these observations to a variety of Xenopus cells and tissues and examines the relationship of nucleosomes to the sequence of 5S DNA. From extensive restriction endonuclease digestion of Xenopus chromatin, no special or phased arrangement of the nucleosomes with respect to one particular site in the 5S genes can be detected.

EXPERIMENTAL PROCEDURES

Purification of Nuclei. Kidney-derived tissue culture cells of X. laevis were grown at 24° in BHK-21 medium (GIBCO) supplemented with 10% fetal calf serum and nonessential amino acids. Cells were dislodged into balanced salts solution and nuclei were isolated as described (20) except the NP-40 concentration was reduced to 0.05% (v/v). Adult female frogs were obtained from Nasco West (Modesto, California). Frogs were anesthetized with 0.1% (w/v) tricaine methanesulfonate (Sigma) and bled by cardiac puncture into 0.15 M NaCl, 5 mM Tris-HCl (pH 7.8), 1 mM EDTA, 2.5 units/ml

heparin. Blood cells were washed twice in this buffer and nuclei were isolated by the method of Van Lente, Jackson and Weintraub (21). Nuclei were also prepared from liver and kidney by this method except the nuclei were sedimented through 1 M sucrose in 10 mM NaCl, 10 mM Tris-HCl, (pH 7.4), 3 mM $MgCl_2$, 0.5% NP-40 three to four times. Nuclei were resuspended in buffer A of Hewish and Burgoyne (22) and digested immediately or stored at -20° in buffer A plus 50% glycerol.

Nuclease Digestions. Micrococcal nuclease (E.C. 3.1.4.7) was purchased from PL Biochemicals and Worthington Biochemicals. All digestions were performed on intact nuclei in buffer A plus 1 mM $CaCl_2$. Nuclei were digested with the restriction enzyme HindIII in buffer A containing 0.2 mM EDTA and 0.2 mM p-chloromercuribenzoate (PCMB) plus 50 mM NaCl and 6 mM $MgCl_2$. Reactions were terminated by the addition of EDTA to 20 mM and SDS to 0.5% (w/v).

Purification of DNA, Gel Electrophoresis and Southern Transfers. SDS-lysed nuclei were treated with proteinase K at 100-200 µg/ml for 1-2 hr at 37°. DNA was purified by extraction with phenol:chloroform:isoamyl alcohol (1:1:0.04, v/v/v) and chloroform:isoamyl alcohol (1:0.04, v/v). The final aqueous phase from these extractions was made 0.3M in sodium acetate and DNA was precipitated at -20° with 2-3 volumes of ethanol. Precipitates were collected by centrifugation at 10,000 g for 10 min, dried under vacuum and dissolved in gel buffer (40 mM Tris-HCl, [pH 7.8], 20 mM sodium acetate, 2 mM EDTA) plus 10% glycerol and 0.05% bromphenol blue. Electrophoresis was carried out in horizontal agarose (Miles, HGT) slab gels (0.5 - 0.8 cm thick). MspI restriction fragments of λgtWES DNA were included in agarose gels as size markers. Fragment lengths were determined by comparison to restriction fragments of pBR322 DNA of known sequence (23). The lengths of the MspI fragments of λgtWES DNA are thus 2300, 2150, 1800, 1030, 691, 575, 530, 541, 438 bp (and smaller fragments of undetermined length). Gels were stained with ethidium bromide (0.5 µg/ml) for 1/2 hr and photographed with UV illumination from below the gel. Denaturation of DNA in situ and transfer to nitrocellulose was carried out as described by Southern (24) with the following modifications. Prior to transfer the nitrocellulose sheets (Schleicher and Schuell, BA85) were soaked in water at 65° for 1 hr. Transfer was in the downward direction with 20X SSC (1X SSC is 0.15 M NaCl, 0.015 M trisodium citrate). After transfer the nitrocellulose sheet was rinsed in 10X SSC, allowed to dry in air for 1 hr and baked in vacuo at 80° for 2-3 hr.

Plasmid DNA and in vitro Labeling. A recombinant plasmid containing a single repeating unit of Xenopus oocyte-type 5S DNA inserted at the HindIII

site of pMB9 and termed pXlo 131 was obtained from Dr. D.D. Brown (Carnegie Institution of Washington, Baltimore, Maryland). Growth and handling of E. coli cells harboring recombinant plasmids was carried out according to the NIH guidelines (P2, EK1). Supercoiled plasmid DNA was purified by CsCl-ethidium bromide buoyant density centrifugation. Plasmid DNA was labeled in vitro by nick translation (25) to a specific activity of 1-3 x 10^7 cpm/μg DNA. DNA polymerase I was obtained from Boehringer-Mannheim and α-^{32}P-deoxyribonucleoside triphosphates (~350 Ci/mmole) were from Amersham or New England Nuclear. DNA was incubated at 10 μg/ml in 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 50 μg/ml bovine serum albumin at 37° with 5 ng/ml DNaseI for 15 min. Unlabeled dNTPs were added to 2.5 μM and one α-^{32}P-dNTP was added to 1 μM. DNA polymerase was added to 25 units/ml and incubation was at 15° for 1-2 hr in a final reaction volume of 50-100 μl. The reaction was terminated by the addition of EDTA to 20 mM and heating to 65°. Unincorporated dNTPs were removed by ethanol precipitation of labeled DNA.

Hybridization. Nitrocellulose sheets were soaked in 3X SSC, 0.2% SDS at 65° for 1 hr and then in modified Denhardt solution containing 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin, 6X SSC, 0.2% SDS for 3 hr at 65°. Hybridization was carried out in this latter solution containing 5-10 ng/ml ^{32}P-labeled pXlo DNA for 16-18 hr at 65°. The nitrocellulose sheets were washed twice at 65° in Denhardt solution as above (without radioactive probe) for 20 min per wash. The sheets were then washed with decreasing concentrations of SSC (3X, 1X, 0.3X) containing 0.2% SDS at 65° for 20 min at each step. Dried filters were subjected to autoradiography with preflashed Kodak XR X-ray film (26) and image intensifying screens (Dupont Cronex).

Quantitation of Southern Transfer Hybridization. In order to determine the relative abundance of 5S gene multimers produced by restriction digestion of nuclei, it was necessary to determine the overall efficiency of Southern transfer and hybridization. To accomplish this a known quantity of MspI restriction fragments of pBR322 DNA was subjected to agarose gel electrophoresis and Southern blot hybridization. The hybridization probe was ^{32}P-labeled pBR322 DNA of known specific activity (labeled by nick translation). After autoradiography the regions of nitrocellulose corresponding to specific bands were cut out and radioactivity was determined by liquid scintillation counting. Fragments of 700-3500 bp gave an overall efficiency of 34-37% while smaller or larger fragments gave progressively

weaker hybridization signals. From this data the weight fraction of hybridization in the 5S gene multimer bands (Figure 6) was normalized.

RESULTS

Xenopus Cells and Tissues have Short Nucleosome Repeat Lengths. The lengths of the DNA fragments produced by digestion of nuclei with micrococcal nuclease were determined for Xenopus liver, kidney, blood and kidney-derived cells maintained in culture. Figure 1 shows the electrophoretic patterns obtained and Table I summarizes the results of several determina-

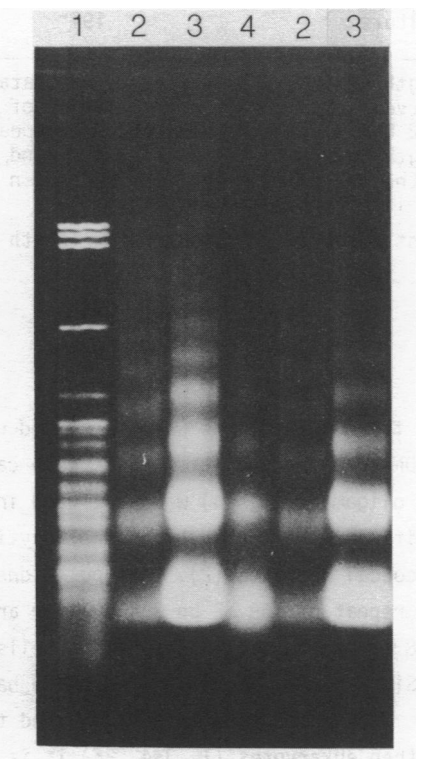

FIGURE 1. Gel Electrophoresis of DNA Fragments Produced by Micrococcal Nuclease Digestion of Xenopus Nuclei.

Nuclei were isolated and digested with micrococcal nuclease (50 units/ml at 37°) for the times indicated below. DNA was analyzed on a 1.7% agarose gel. (1) λgtWES DNA digested with MspI; (2) Cultured kidney cell nuclei, 1 min; (3) Blood cell nuclei, 30 sec; (4) Liver nuclei, 5 min. Times were chosen to show comparable extents of digestion.

TABLE 1. Nucleosome Repeat Lengths of Xenopus Chromatin and Chromatin from Other Sources.

Cell Type	DNA Repeat Length (bp)[a]
Xenopus blood	184 ± 4 (9)[b]
liver	178 ± 4 (9)
kidney	178 ± 2 (6)
kidney cells in culture	169 ± 2 (5)
Chicken erythrocytes	207 - 212[c,d]
liver	202[d]
Rat liver	196[c]
kidney	196[c]
kidney primary culture	191[c]

[a] Determined from length of DNA at band center from data similar to Fig. 1 using a standard curve constructed from mobilities of restriction fragments of λgtWES and pBR322 DNA. For each band the DNA repeat was then calculated by dividing the length of DNA in the band by the band number. The mean of repeat lengths was then calculated for multimers of n > 3. The repeat length is shown along with its standard deviation.

[b] Number of independent determinations of repeat length shown in parentheses.

[c] From Compton et al., 1976.

[d] From Morris, 1976.

tions. The lengths of the DNA fragments were estimated using restriction fragments of λ and pBR322 DNA (23) and repeat lengths were calculated from these data (Table 1). Small oligomers (n < 4) were not used in this analysis as their lengths are significantly affected by exonucleolytic attack by the continued action of micrococcal nuclease (2). Xenopus kidney cells in culture thus have a nucleosome repeat of 169 ±2 bp while liver and kidney have repeats of 178± 4 and 178± 2 bp, respectively. Blood cells have a nucleosome repeat of 185± 4 bp. Similar data for liver and blood have been reported by Humphries et al. (15). When these results are compared to the DNA repeat lengths reported for other eukaryotes (1, 3-4, 26) it is evident that Xenopus cells have shorter nucleosome repeats than corresponding cells and tissues from other higher organisms (Table 1). This is particularly striking for blood cells. Avian erythrocytes have a repeat of 207-212 base pairs (3,26) while Xenopus nucleated blood cells have a repeat length some 25 bp shorter. Nevertheless, the relative differences in repeat lengths for Xenopus tissues and cells are similar to those differences in other animals (Table 1, ref. 15).

Nucleosome Repeat of 5S Gene Chromatin. The micrococcal nuclease cleavage pattern of a particular DNA sequence in chromatin can be revealed using the blotting technique of Southern (24) in conjunction with a radioactive DNA or RNA probe for that sequence (9, 15, 27-28). Using a recombinant plasmid containing a single repeating unit of Xenopus oocyte-type 5S DNA (pXlol31, obtained from Dr. D.D. Brown) I have determined the repeat length of the chromatin regions containing the 5S genes. As oocyte-type 5S genes represent the majority of 5S DNA sequences in the Xenopus genome, the hybridization patterns reflect the organization of oocyte-type genes in chromatin. Figure 2 illustrates the autoradiogram for the hybridization of ^{32}P-labelled pXlo DNA to the DNA fragments produced by micrococcal nuclease digestion of blood nuclei. Note

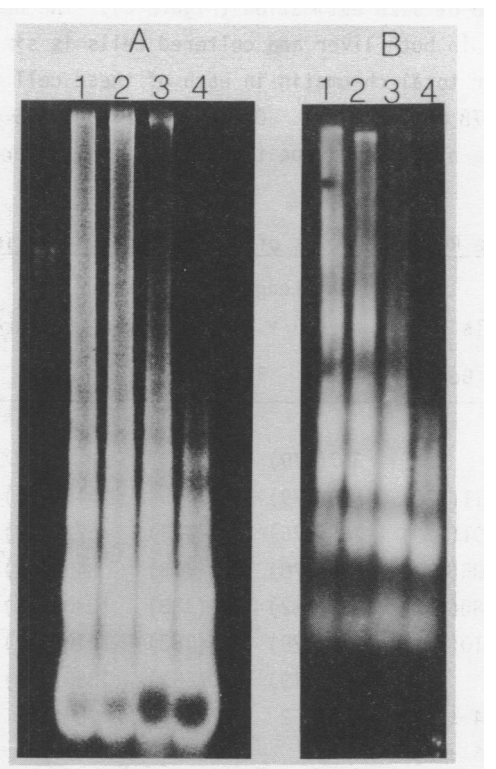

FIGURE 2. Micrococcal Nuclease Digestion Pattern of 5S Gene Chromatin.

Blood nuclei were incubated with 50 units/ml of micrococcal nuclease for (1) 30 sec; (2) 1 min; (3) 2 min; and (4) 5 min at 37°. DNA was prepared and subjected to electrophoresis in a 1.5% agarose gel. (A) Ethidium stain; (B) Southern blot hybridization to pXlo ^{32}P-DNA.

that the monomer band is not retained on nitrocellulose in this experiment. Also note that at the earliest times of digestion the usual nucleosome multimer pattern is present in the hybridization autoradiogram only up to the hexamer; above this point a different series of bands is seen. These bands of hybridization occur at intervals of about 4 nucleosomes (8,12,16..,) or every 700 bp. This latter value corresponds to the DNA sequence repeat of oocyte-type 5S DNA (16-18). This finding is suggestive of a higher order structure in 5S gene chromatin. This is the subject of current investigation (Gottesfeld and Bloomer, in preparation). Nucleosome repeat lengths have been calculated from these data and from additional experiments of this type (Table 2). Restriction fragments of λ DNA were included in the Southern blots for the purpose of size estimation (Figure 2). The nucleosome repeat of 5S gene chromatin in both liver and cultured cells is similar to the average repeat length for total chromatin in each of these cell types (165 bp in cultured cells and 178 bp in liver). On the other hand, 5S gene chromatin in blood nuclei has a nucleosome repeat some 5-10 bp shorter than the aver-

TABLE 2. Nucleosome Repeat Lengths of 5S Genes in Chromatin.

	DNA Repeat Lengths (bp)					
	Blood Cells		Liver		Cultured Cells	
Band No.	Total[a]	5S Genes[b]	Total	5S Genes	Total	5S Genes
1						
2			357(179)			
3	560(187)[c]	511(170)	538(179)		515(172)	498(166)
4	740(185)	691(173)	705(176)	692(173)	672(168)	655(164)
5	910(182)	885(177)	890(178)	863(173)	845(169)	826(165)
6	1110(185)	1080(180)	1090(182)	1090(182)	1030(172)	994(166)
7	1295(185)	1210(172)	1250(179)	1275(182)	1210(172)	1179(168)
8	1490(186)		1430(179)	1450(181)	1355(169)	
Mean	185± 2	174 ± 4	179 ± 2	178 ± 5	170 ± 2	166 ± 2
Mean[d]		176 ± 2				165 ± 2

[a] From ethidium stained gels similar to Figure 1.

[b] From hybridization to pXlo ^{32}P-DNA; data given for earliest digestion times.

[c] Band length in bp estimated from restriction fragments of λgtWES and pBR322 DNA. Repeat length (band length divided by band number) in parentheses.

[d] Average repeat length ± S.D. for five independent determinations.

age nucleosome repeat. In each of five separate determinations a shorter 5S gene nucleosome repeat has been obtained for blood nuclei. Similar results have been obtained by Humphries et al. (15) for liver and blood nuclei.

The results with blood nuclei demonstrate that the nucleosome repeat on a particular DNA sequence can differ from the average repeat in the cell nucleus. Further, the results with cultured cells show that the nucleosome organization of a given gene can differ from one cell type to another. It is possible, however, that the difference in nucleosome repeat of the 5S genes (and of the average repeat as well) observed with cultured cells could be due to some unusual property of cells maintained in culture (see ref. 26). It will be of interest to see whether other genes in chromatin exhibit properties similar to those of the 5S genes.

Restriction Endonuclease Cleavage of 5S Genes in Chromatin. The relationship between nucleosomes and the sequence of 5S DNA can be investigated using restriction endonucleases and the Southern blotting technique. Specifically, I wish to distinguish between random and non-random alignment of nucleosomes with respect to a unique restriction site in 5S DNA. There is one HindIII site per sequence repeat in oocyte-type 5S DNA (16, 18). Therefore, nuclei from blood, liver and cultured cells have been incubated with HindIII and the products of digestion have been analyzed on agarose gels. The ethidium bromide staining pattern of HindIII-cleaved Xenopus DNA (Figure 3A, lane 1) reveals a broad distribution of DNA fragment lengths. In addition, one prominate band at 700 bp is seen. Although similar in length to 5S DNA, the 700 bp band is not 5S as judged by several criteria - band sharpness, different position in buoyant density gradients and lack of hybridization to 5S RNA (B. Lam and D. Carroll, personal communication). In a HindIII digest of blood cell nuclei multimers of the 700 bp band are seen (Figure 3A, lanes 2-4). This indicates that these sequences are organized in tandem repeating units in the Xenopus genome and that some of the HindIII sites are protected from enzyme cleavage by chromatin proteins.

Figures 3B and 4 illustrate the hybridization of ^{32}P-pXlo DNA to blots of these HindIII digests. When purified DNA is incubated with HindIII, greater than 90% of the repeating units are converted to the monomer 690 bp species (Figure 3B. lane 1). The broadness of the bands of 5S hybridization is due to sequence heterogeneity in the oocyte-type 5S DNA; individual 5S repeats differ in their content of 15-nucleotide internal repetitious sequences (16-18). When chromatin is digested in situ, a series of bands of hybridization are observed. These bands are integral multiples of the repeating unit. This par-

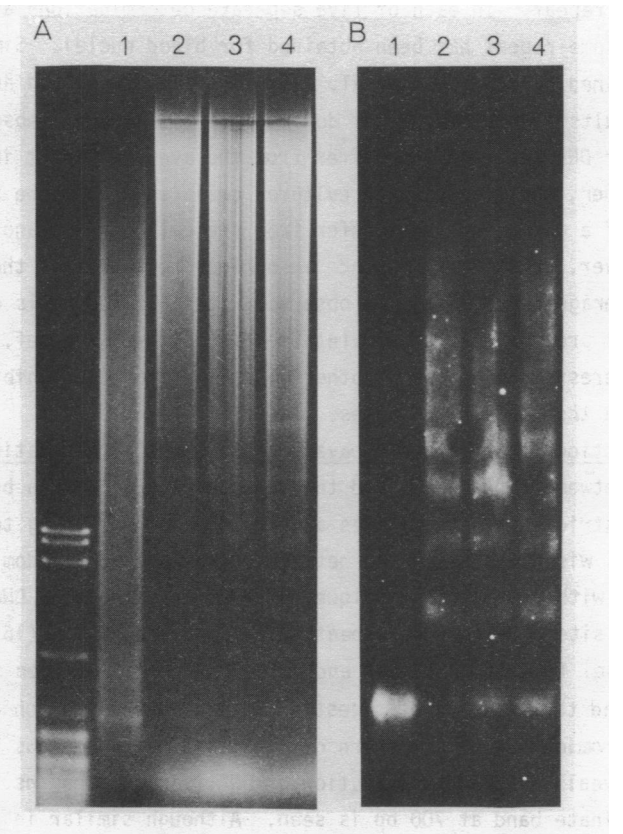

FIGURE 3. HindIII Cleavage of Blood Nuclei.

Blood DNA (1) or nuclei (2-4) at 10 µg DNA per sample were incubated at 37° for 1 hr with (1) 2; (2) 25; (3) 15; (4) 10 units/µg DNA of HindIII. DNA was isolated and subjected to electrophoresis on a 0.6% agarose gel. (A) Ethidium stained gel. (B) Hybridization autoradiogram of Southern blot with pXlo ^{32}P-DNA probe. MspI restriction fragments of λgtWES DNA are also shown in A.

tial cleavage pattern reflects the tandem organization of 5S genes in the Xenopus genome and indicates that some HindIII sites in 5S gene chromatin are protected from enzyme digestion. Even with large amounts of enzyme (80 units/µg of DNA) a significant fraction of HindIII sites are not cleaved. Repeated additions of HindIII or longer incubation times did not change the chromatin digestion pattern. Furthermore, addition of λ DNA near the end of the incubation resulted in complete digestion of this DNA - showing that the enzyme

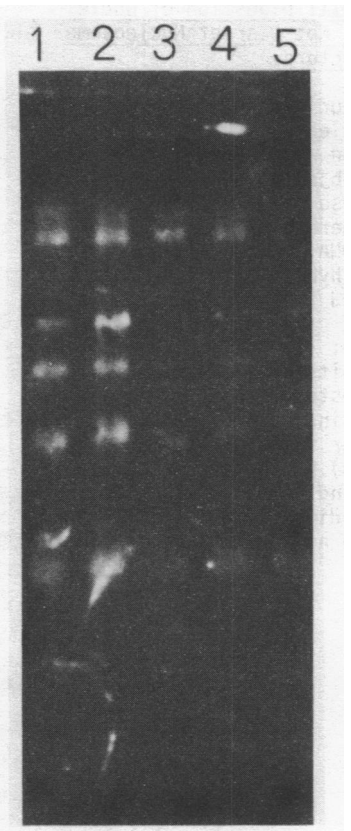

FIGURE 4. HindIII Cleavage of 5S Genes in Cultured Cell Nuclei.

Nuclei (5 μg DNA per sample) were incubated at 37° for 2 hr with (1) 40; (2) 30; (3) 20; (4) 10; (5) 2 units/μg DNA of HindIII at 37°. DNA was isolated and subjected to electrophoresis on a 1.5% agarose gel. The hybridization autoradiogram of a Southern blot is shown. The probe was pXlo ^{32}P-DNA.

remained active. These results demonstrate that a true limit digest had been achieved and that the enzyme was not inactivated by the nuclear preparation or by the assay conditions. These results support the conclusion that some HindIII sites in 5S gene chromatin are not available for cleavage.

In order to interpret these data with respect to nucleosome organization on the 5S genes, it is important to demonstrate that HindIII digestion of nuclei does not disrupt nucleosome structure. This has been accomplished by sequential digestion of nuclei with HindIII and micrococcal nuclease. Figure 5 shows the electrophoretic patterns of total chromatin DNA (ethidium stained pattern) and 5S gene chromatin (blot hybridization with ^{32}P-pXlo DNA) for nuclei digested with micrococcal nuclease both with and without prior digestion with HindIII. As expected the average length of both total and 5S gene chromatin DNA fragments is reduced by HindIII digestion (Figure 5,

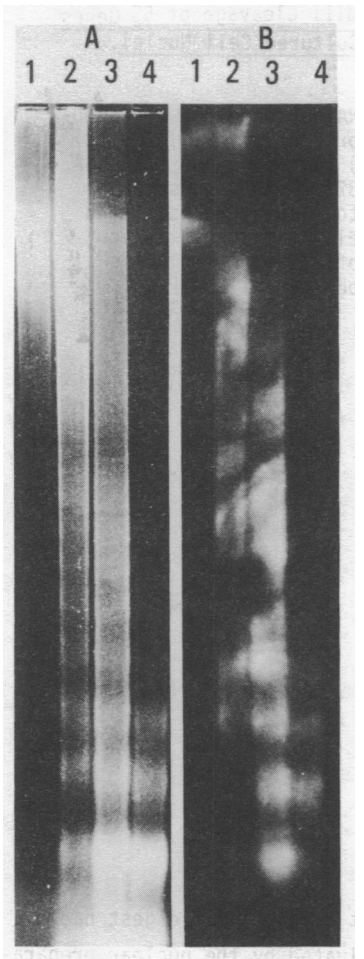

FIGURE 5. HindIII Digestion of Nuclei Does Not Disrupt Nucleosome Structure.

Cultured cell nuclei were incubated with micrococcal nuclease with or without prior incubation with HindIII. DNA was isolated and subjected to electrophoresis in a 1.7% agarose gel. 5S DNA was detected by Southern blot hybridization with pXlo ^{32}P-DNA. (A) Ethidium stained gel. (B) Blot hybridization. Note that the monomer band is not retained on nitrocellulose. (1) Nuclei incubated without enzymes; (2) Micrococcal nuclease digest at 25 units/ml for 1 min at 37°; (3) Nuclei digested with micrococcal nuclease after incubation in HindIII digestion buffer for 5 hr at 37° without HindIII; (4) Nuclei incubated with 1.6 units of HindIII per µg DNA for 5 hr at 37° then digested with micrococcal nuclease at 25 units/ml for 1 min.

lane 4); however, integrity of the nucleosome bands and repeat lengths are not affected by HindIII digestion.

Experimental data on the partial cleavage of polymers have been analyzed mathematically by Kuhn (29) and more recently for restriction cleavage of DNA by Botchan, McKenna and Sharp (30) and by Manteuil, Hamer and Thomas (31). From these analyses, the relative amount of DNA in each multimer band on a gel can be predicted for a given random fraction of protected restriction enzyme recognition sites. If a random fraction (p) of all sites are protected, the weight fraction (w) of DNA in each multimer band (n) will be

$$w_n = n(1-p)^2 p^{(n-1)}.$$

When the weight fraction of hybridized DNA in each band divided by band number was plotted against (n-1) a straight line was obtained (Figure 6). The slopes indicate that for blood, liver and cultured cell nuclei about 43% of the sites are protected. That is, in a limit digest 57% of all HindIII sites in 5S gene chromatin can be cleaved. Since the relative abundance of 5S gene multimers follows a Kuhn distribution, the data indicate that the distribution of available HindIII sites is random. This suggests that there is no special or phased arrangement of the nucleosomes on 5S DNA.

The fraction of available HindIII sites in 5S gene chromatin (57%) strongly suggests that this restriction endonuclease cleaves DNA in both nucleosome core and linker regions. A calculation of the expected

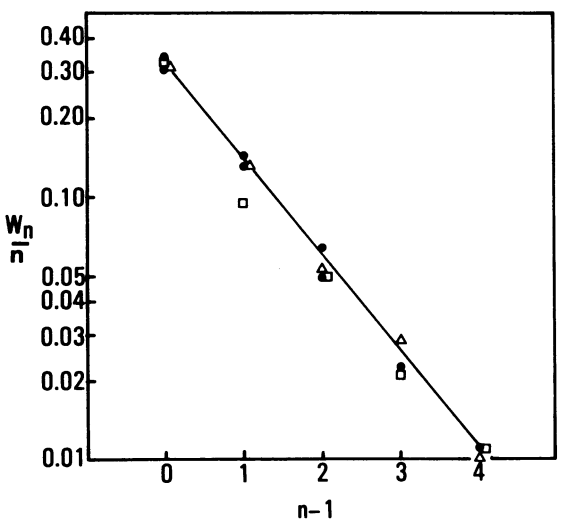

FIGURE 6. Fraction of Accessible HindIII Sites in 5S Gene Chromatin.

The weight fraction of 5S gene hybridization in each multimer band (w_n) divided by band number (n) is plotted against (n-1). w_n was estimated by cutting the bands from dried Southern blot nitrocellulose sheets (similar to those whose autoradiograms are shown in Figures 3 and 4) and counting with a toluene-based fluor. Locations of bands was determined from the autoradiograms. Data for (o) cultured cells (two experiments); (Δ) blood; ( ) liver nuclei. In a control experiment it was found that Southern transfer and hybridization were equally efficient for DNA fragments of 700-3500 bp (data not shown). The fraction of accessible sites was 57% for cultured cells and liver and 59% for blood.

fraction of monomer length 5S DNA arising from cleavage only in linker regions illustrates this point. The mean length of 5S monomer DNA is 690 bp with a standard deviation of 9% or 63 bp (18). If cleavage takes place only in linkers, monomers will arise from DNA spanning from four cores plus three linkers to four cores plus five linkers. For liver or blood chromatin this will be 674 to 734 bp. From the normal distribution function, the fraction of all 5S monomers whose length ranges from 674 to 734 bp is 0.36. The probability of a HindIII hexanucleotide recognition site lying solely in a linker region is 0.136 (linker length - 6 divided by repeat length equals 40-6/176).Thus the probability of two adjacent HindIII sites both lying in linker regions is 0.05 (0.136 x 0.36). Clearly the fraction of 5S hybridization to the monomer band (Figures 3, 4, 6) is far greater than five percent. Thus it is likely that HindIII can cleave DNA both on the nucleosome core and in the linker region.

DISCUSSION

In the higher eukaryotes variations exist in nucleosome repeat lengths between different species and between cell types in the same organism. Compton et al. (26) and many others (see ref. 1 for references) have found a repeat of about 196-200 bp for the cells of mature tissues; smaller values are found for cells of actively dividing tissues and cells in culture. No general correlation, however, can be made between repeat length and rate of cell division. Transcriptionally active cells appear to have shorter repeat lengths than transcriptionally inactive cells (31, 32) but exceptions to this rule exist (33). Since histone H1 is thought to lie in the variable-length linker region of the nucleosome (2), Noll (4) and Morris (3) have proposed that differences in H1 histones are responsible for variations in linker lengths. In each case examined, Xenopus cells had shorter repeat lengths than the corresponding cells in other higher animals (Table 1 and ref. 1); however, the relative differences between the cell types of Xenopus are similar to those differences in other organisms. The origin of short nucleosome repeats in Xenopus cells and tissues is at present unknown. Xenopus core histones H2B, H3 and H4 have electrophoretic mobilities similar to those of the core histones of calf, while Xenopus H2A migrates slightly slower than calf H2A (34). Xenopus cultured cells have several species of H1 histones and Xenopus erythrocytes contain additional polypeptides in the H1 region. These latter proteins may correspond to the avian histone H5. Thus Xenopus

possesses the usual complement of histones.

In liver and kidney cells maintained in culture the nucleosome repeat of the 5S genes is similar to the average nucleosome repeat for the bulk DNA in these cells. On the other hand, in blood cells the 5S gene nucleosome repeat is significantly shorter than the repeat for the bulk DNA. Further, the nucleosome repeat for 5S DNA in cultured cells (165 ± 2 bp) is shorter than the repeat for these genes in blood and liver (176 - 178 bp). In previous studies (9) no differences were found between the average nucleosome repeat for the bulk DNA and specific sequences such as mouse satellite DNA and the DNA sequences coding for 18 and 28S ribosomal RNA. The present results and those of Humphries et al. (15) clearly demonstrate that (i) a particular DNA sequence can have a nucleosome repeat which is distinct from the average repeat in the cell nucleus and (ii) that the nucleosome repeat of a given gene can differ between cell types.

If there is a precise phased relationship between the DNA sequence of the 5S genes and nucleosome position on these genes, then a specific sequence that is available for restriction enzyme cleavage in one 5S DNA repeat will be available in all repeating units. Conversely, a site which is not accessible in one repeat will not be accessible in any of the repeats. Thus if phasing occurred in 5S gene chromatin, either all 5S repeats will be recovered as 700 bp fragments after digestion with a single site restriction enzyme or no cleavage will occur. For a random arrangement of nucleosomes, restriction enzyme cleavage should yield a series of 5S DNA fragments of different multimer lengths. From an analysis of the products of extensive restriction endonuclease cleavage of 5S gene chromatin no special or phased arrangement of the nucleosomes can be detected. HindIII cleavage produces a series of multimers of 5S DNA whose relative abundance follows a Kuhn distribution (Figure 6). Since sequence and length heterogeneity exists in the major oocyte-type 5S genes, precise phasing of the nucleosomes with respect to individual copies of the 5S genes in each cell nucleus could exist and would not be detected by the methods employed. It is also important to note that if nucleosome sliding occurred during nuclease digestion precise phase relationships might have been obscured. Control experiments, however, did not detect any significant alteration of nucleosome structures during restriction endonuclease digestion (Figure 5); however, it is not possible to rule out nucleosome sliding in isolated nuclei from these types of experiments.

The question of nucleosome phasing has now been addressed by many authors. For nonrepeated DNA sequences, Prunell and Kornberg (35) have demonstrated that the location of nucleosome cores is random. Similarly, Garel and Axel (36) suggest that the nucleosomes on the chicken ovalbumin gene are random. For repetitive sequences, Singer (27),Fittler and Zachau (37) and Lipchitz and Axel (6) find no evidence for phasing of nucleosomes on satellite DNA. On the other hand, Brown et al. (38) have reported a precise phase relationship between nucleosomes and satellite DNA sequences. These results, however, could be due to sequence-specific cleavage of satellite DNA by micrococcal nuclease (37). Taken together the results appear to rule out precise phasing of nuclesomes and DNA sequences; however, the possibility of nucleosomes occupying a number of distinct alternative positions has not been eliminated (39-40).

In a limit digest 57% of the HindIII sites in 5S gene chromatin are cleaved by this enzyme. Control experiments showed that this was indeed a limit digest; the enzyme remained active near the end of the incubation and additional enzyme did not change the digestion profile. Thus chromatin proteins block 43% of the HindIII sites from cleavage. From a calculation of the expected yield of monomer length 5S fragments one conclusion seems firm: HindIII must cleave DNA in both the nucleosome core and linker regions. Heterogeneity may exist in 5S gene chromatin such that some nucleosomes block HindIII cleavage while others do not. Perhaps the exact position of a given nucleotide sequence on the nucleosome core dictates its availability for cleavage. This suggestion is supported by the finding that the nucleosome core is not homogeneous in its nuclease sensitivity (41). Alternatively, the higher order structure of chromatin may dictate which HindIII sites are available for cleavage. It is interesting that although the nucleosome repeat length of 5S gene chromatin is different for cultured cells and liver or blood nuclei, the fraction of available HindIII sites is the same in each of these cell types. Furthermore, Lipchitz and Axel (6) have found the same percentage (57%) of EcoRI sites of calf satellite chromatin available for cleavage. The large fraction of available restriction endonuclease sites in chromatin suggests that nucleosomes might not act as barriers for the sequence specific recognition of DNA by regulatory proteins or polymerases.

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