Volume 8 Number 22 1980 Nucleic Acids Research

Non-random arrangement of nucleosomes in satellite I containing chromatin of rat liver

Tibor Igo-Kemenes, Akira Omori and Hans G.Zachau

Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, GFR

Received 23 June 1980

ABSTRACT

The location of nucleosomes on the nucleotide sequence of rat satellite I DNA was investigated using micrococcal nuclease, exonuclease III, and restriction nucleases as tools. Hae III cleaved the satellite DNA containing chromatin very preferentially in the linker region. Nucleosomes were found predominantly in three defined positions on the 370 bp satellite I monomer unit. This type of arrangement occurs on not more than half of the satellite DNA containing chromatin while the rest of this chromatin is arranged differently. The arrangement of nucleosomes with high probability in preferred frames and with low probability in less preferred frames may be a general phenomenon which can be discussed as a possible mechanism to modulate sequence recognition.

INTRODUCTION

The problem of specific vs random location of nucleosomes on DNA has been widely studied in recent years. Depending on the systems and the methods employed different results were obtained. While in early reports on viral systems a random location was found (1,2), more recent reports using refined methods revealed sites of preferential location of nucleosomes (3), sites of high nuclease susceptibility devoid of nucleosomes (4-6) and sites with high affinity for nucleosomes in reconstitution studies (7). Experiments on random cellular DNA of rat liver (8) and on genes coding for 5S RNA (9) indicated a random location of nucleosomes. Preferential locations of nucleosomes on certain DNA sequences were reported for chicken tRNA (10) and Xenopus 5S RNA genes (11). Specific arrangements were also found on a Drosophila heat shock gene (12) and on the constant gene region of the immunoglobulin kappa light chain of the mouse (13). For the α-satellite DNA containing chromatin of African green monkey cells a unique

phase relationship between nucleosomes and the DNA was proposed (14). But the conclusions of this publication were questioned by other authors (15-17). While a unique relationship between DNA sequence and nucleosome location was practically excluded it was specifically stated (17) that more complicated phase relationships may be operative also in the α-satellite DNA containing chromatin.

In the preceding paper (18) we report that in the rat satellite I DNA containing chromatin the nucleosome repeat length (185 bp) is just half of the DNA repeat length which had previously been determined (19) to be 370 bp. This coincidence raised the possibility of a specific, though not necessarily simple, phase relationship between nucleosomes and satellite I DNA. Experiments pertinent to this question are reported in the following.

MATERIALS AND METHODS

Materials and methods were as in the preceding paper (18) except for the following.

Exonuclease III treatment of solubilized chromatin. Micrococcal nuclease treated nuclei were washed as described (20) and pelleted. Lysis of the nuclei and extraction of soluble chromatin with 1 ml buffer per 150 µg of nuclear DNA was performed under vortexing for a few seconds with 10 mM Tris-HCl, pH 7.9, 0.2 mM EDTA, 0.02 mM EGTA, 1 mM PMSF at 0^{o} and occasional gentle agitation for further 30 min. Solubilized chromatin (1.5 A_{260} unit/ml) was supplemented with 1.2 mM $MgCl_2$, 66 mM Tris-HCl, pH 8, 1 mM ß-mercaptoethanol. This step was performed by adding a 10 times concentrated buffer solution under vortexing followed by the addition of 5.5 units/ml exonuclease III (New England BioLabs, Beverly MA 01915). During buffer addition the chromatin solution became slightly turbid due to chromatin aggregation in the presence of Mg^{2+}. Care was taken to get the suspensions as homogeneous and dispersed as possible. Incubation was for 45 min at 37^{o} with occasional shaking. The reaction was terminated by the addition of 2 mM EDTA, and ethanol precipitation. The ethanol precipitate was deproteinized by incubation with proteinase K at a DNA concentration of 250 µg/ml. Proteinase K and

SDS were removed by three ethanol precipitations and the DNA finally dissolved in 10 mM Tris-HCl, pH 7.6 (without EDTA).

S1 nuclease treatment of exonuclease III trimmed DNA. Exonuclease III treated DNA was incubated at 20° for 30 min in a buffer containing 200 mM NaCl, 25 mM potassium acetate, pH 4.5, 0.1 mM $ZnSO_4$, 1.25 units/ml S1 nuclease (21), at a DNA concentration of 30 µg/ml. Reaction was terminated by the addition of 100 mM Tris-HCl, pH 8.8, followed by ethanol precipitation. DNA was dissolved at a concentration of 500-1500 µg/ml in the buffer containing 50 µg/ml proteinase K. After incubation, 5-10 µg aliquots were directly applied to agarose gels.

Hae III digestion of rat liver nuclei. This was performed similarly to ref. (20) using Hae III concentrations of 1000 units/ml and 1 mg nuclear DNA/ml during 30 min of digestion at 37°. Termination of digestion, washing of nuclei and chromatin extraction were as described for the micrococcal nuclease digestion experiments (see above).

RESULTS

Analysis of the length variability of the linker DNA between nucleosome cores on satellite I DNA. The numerical identity of the nucleosomal repeat length of satellite chromatin with half of the satellite DNA repeat would allow for a direct relationship between location of the nucleosomes and the DNA sequence if the linker lengths were constant. It was therefore of interest to investigate possible variations in linker length. One approach to detect length variations of linker DNA was outlined by Prunell and Kornberg (8) who used a combination of micrococcal nuclease, exonuclease III, and S1 nuclease digestions for this purpose. A partial micrococcal nuclease digest of chromatin was prepared and oligonucleosomes were trimmed by the action of exonuclease III and S1 nuclease. Since the oligonucleosomal bands, particularly the dinucleosomal one, were shortened but not sharpened it was concluded that the linkers vary in length. In our analogous experiments we wanted to detect the distribution of satellite DNA lengths within the bulk DNA of a micrococcal nuclease digest and therefore had to use blotting and hybridization techniques.

The result of such an experiment is shown in Fig. 1. The action of exonuclease III and S1 nuclease led to increased mobilities of the nucleosomal DNA fragments. The mononucleosome (185 bp) was trimmed to a sharp 145 bp fragment, corresponding to the size of the core DNA. The dinucleosomal DNA was clearly shortened but the width of the band was virtually unchanged. In higher oligonucleosomes the resolution of the gel was not sufficient to allow a clear interpretation. In control incubations in which either exonuclease III or S1 or both nucleases were omitted no change in mobility or width of the mono- or dinucleosomal bands was detected.

These findings practically exclude, as in the case of the African green monkey satellite fraction (17), a perfect and simple phase relationship between the nucleosome and the satellite I DNA periodicities over a long range. But the findings do not allow the conclusion that the nucleosomes are randomly distributed on the satellite I DNA. A complex, but still specific arrangement of nucleosomes is not excluded.

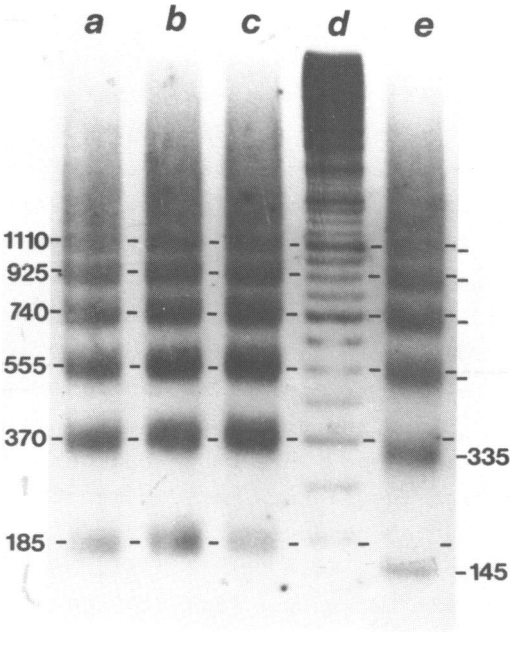


Figure 1. Demonstration of length heterogeneity of nucleosome spacing in satellite I containing chromatin. Chromatin fragments generated by micrococcal nuclease digestion of nuclei were trimmed with exonuclease III and S1 nuclease and the DNA subjected to gel electrophoresis on a 1.5 % agarose slab gel, track e. A partial EcoRI digest of enriched satellite I DNA is shown in track d for chain length markers. The wells a-c were loaded with DNA from control samples where S1 nuclease (c), exonuclease III (b), or both nucleases (a) were omitted from the incubation mixture. Hybridization patterns with ^{32}P-labelled satellite I DNA are shown.

Histone cores are found in specific positions of the 370 bp fragment generated by Hae III digestion of rat liver nuclei. The use of a restriction nuclease for chromatin solubilization instead of non-specific endonucleases is advantageous since a defined subset of oligonucleosomes is generated due to selection for accessible restriction sites located in the linker regions.

The location of terminal nucleosomes on a DNA fragment or on a series of separable DNA fragments can be determined by experiments in which the protruding ends are trimmed by exonuclease III and S1 nuclease digestion. If the nucleosome cores were located on the DNA fragment(s) in a random fashion a broad band of shortened fragments would be expected. In case of specific location(s) one or several sharp bands should be observed. Such an approach is possible since it was shown that exonuclease III alone (22) or in combination with S1 nuclease (8) can remove from mono- and oligonucleosomes, produced by endonucleolytic cleavage of chromatin, the terminal DNA segments without invading the core itself. In Fig. 2 the exonuclease/S1 experiment is shown and in Fig. 3 a subsequent Hind III experiment by which the trimmed sites were mapped. The strategy of the experiments and the results are summarized in Fig. 4.

Chromatin was solubilized from nuclei by Hae III digestion and treated with exonuclease III. After deproteinization single stranded tails which had been formed by the exonuclease III treatment were removed with S1 nuclease. The trimmed DNA fragments were subjected to gel electrophoresis and the distribution of satellite fragments within the bulk of the digested material was revealed by blotting and hybridization (Fig. 2). For the dinucleosome one would expect, in case of a random location of the cores, a broad band between 290 bp (two closely spaced cores) and 370 bp; analogously broad bands would be derived from the oligonucleosomes. What we found, however, were fairly narrow bands centered around 335 bp, 705 bp etc. (track d in Fig. 2). This result indicates that only a small number of fragments were generated. A measure for the band width can be taken from track h where a DNA sample without exonuclease III treatment was added to the trimmed sample and coelectrophoresed; the shortened and the original 370 bp fragments were well separated with negligible

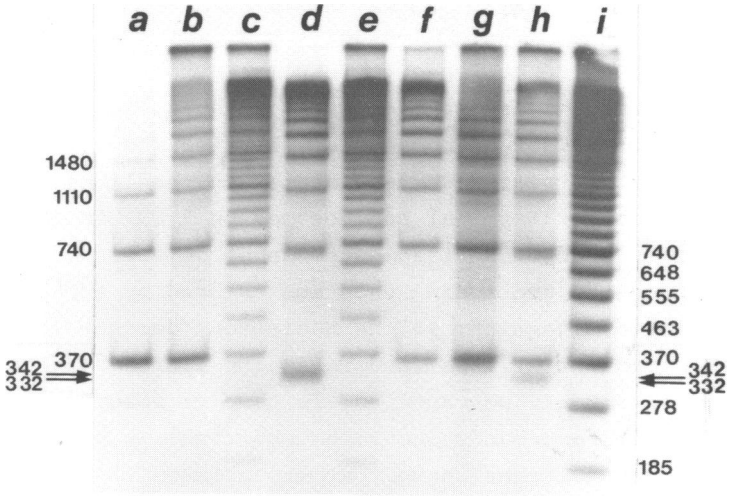


Figure 2. Exonuclease III and S1 nuclease treatment of Hae III generated satellite I containing chromatin fragments revealed that nucleosomes on these fragments occupy only a limited number of positions. Gel electrophoresis was on a 1.2 % agarose slab gel. After electrophoresis the DNA was transferred to nitrocellulose and hybridized with ^{32}P-labelled satellite I DNA. An autoradiogram of the blot is shown. Track a: a late partial Hae III digest of rat DNA served as chain length marker. Tracks c,e, i: a partial EcoRI digest of enriched satellite I DNA provided additional chain length markers; 0.5 µg (c,e) and 2 µg of DNA (i) were applied. Track b: chromatin was prepared by Hae III digestion and subsequent extraction of rat liver nuclei; 5 µg of the DNA were applied. Track d: chromatin as in track b but treated with exonuclease III, proteinase K and S1 nuclease under the conditions of ref. 3; 5 µg of DNA were applied. Tracks f,g: control samples, where exonuclease III or S1 nuclease, respectively, was omitted. Track h: DNA from tracks b and d were mixed and coelectrophoresed.

background between the two bands. In some gels the shortened band of ca. 335 bp was resolved as a double band centered around 332 and 342 bp. No significant changes were detected in control samples where exonuclease III or S1 nuclease had been omitted from the incubations. The limited resolution of the gel in the higher molecular weight region did not allow clear conclusions concerning the multimers of the 370 bp unit fragment.

In order to determine the extent of trimming on either side of the dinucleosome fragment we have eluted the exonuclease III

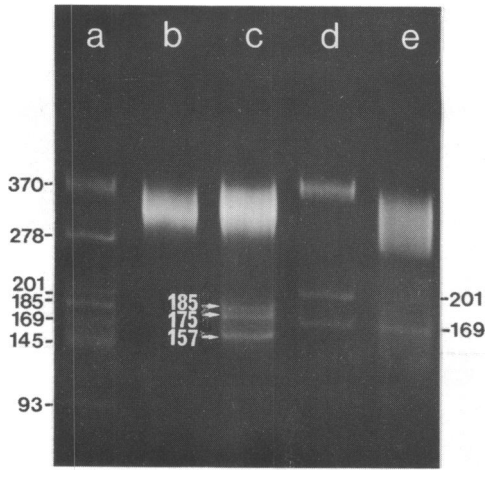


Figure 3. Nucleosomes on the 370 bp satellite fragment are located prodominantly in three specific positions. Samples of chromatin, prepared by Hae III digestion of nuclei, were trimmed with exonuclease III/S1 nuclease before and after removal of histones with proteinase K. Samples of trimmed and untrimmed material were subjected to electrophoresis on preparative agarose gels. DNA from the 370 bp region of the untrimmed and from the 290-370 bp regions of the trimmed material were eluted from the gel, digested with Hind III, and subjected to electrophoresis on a 2 % agarose slab gel. Track a: chain length markers obtained by mixing isolated restriction fragments from rat satellite I (92/93, 185 and 277/278 bp fragments from EcoRI digests) with 169 and 201 bp fragments from Hind III/Hae III digests as in track d and the 146 bp core fragment derived from an exonuclease III/S1 digest of rat mononucleosomes. Track b: trimmed DNA without Hind III digestion. Track c: Hind III digest of the DNA sample trimmed in the presence of nucleosomes. Track d: Hind III digest of the untrimmed DNA. Track e: Hind III digest of the DNA sample trimmed after removal of nucleosomes. The bands were visualized by ethidium bromide staining of the gel.

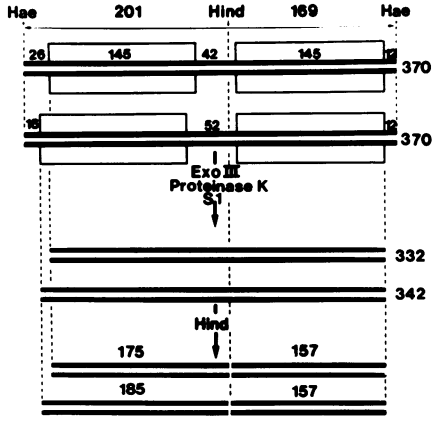


Figure 4. Localization of nucleosome cores on the 370 bp Hae III fragment from satellite I containing chromatin. The strategy of the experiments and the interpretation of the results of Fig. **2** and **3** are shown schematically. DNA is depicted as double bar and nucleosome cores as boxes. Details are discussed in the text.

and S1 nuclease digested DNA from the 290-370 bp region of a preparative agarose gel and further digested with Hind III (Fig. 3). Since the Hind III site is located asymmetrically on the 370 bp fragment (see Fig. 4) one can thus determine which of the two termini was trimmed to what extent. The satellite I DNA was converted predominantly into three sharp bands with chain lengths of 157, 175 and 185 bp, in relative amounts of 1:0.7:0.3, respectively (track c of Fig. 3). Some additional faint bands were neglected in this quantitation. Most of the DNA, which was not satellite DNA, was cleaved by Hind III only to a small extent and remained at its original position on the gel.

Our results are summarized and interpreted in Fig. 4. Nucleosomes on the Hae III generated 370 bp fragment were found to be located in two alternative positions on the 201 bp Hae-Hind arm and in only one position on the 169 bp Hind-Hae arm of the fragment. The dinucleosomes with the 26 and 12 bp protruding ends comprise 67 % of the population while the ones with the 16 and 12 bp termini are present in 33 %. The respective linker lengths are inferred to be 42 and 52 bp in two types of dinucleosomes. The location of the nucleosome cores can be stated with a certainty of about $\pm$ 5 bp which is the limit of accuracy in our length determinations of the 157, 175 and 185 bp fragments.

These results are meaningful only if the exonuclease is stopped by the nucleosome cores themselves and not by peculiarities of the DNA sequence. Two types of control experiments were done to exclude that the results are due to retardation sites in the DNA sequence. In one set of experiments Hae III prepared chromatin was first deproteinized and the DNA digested to various extents with exonuclease III. The single stranded 3'-tails were then removed with S1 nuclease. The trimmed DNA samples were subjected to preparative gel electrophoresis and the fragments from the 290-370 bp region cleaved with Hind III and again electrophoresed.

In the second set of experiments a Hae III digest of purified (19) rat satellite I DNA was treated analogously with exonuclease III, S1 nuclease and Hind III. The results of the first set of experiments are documented only by showing the electropherogram of one Hind III digest (Fig. 3e) while several digests

are shown from the other group of experiments (Fig. 5). In both sets of experiments on exonuclease III/S1 nuclease treatment the 370 bp band became shorter and more diffuse and eventually disappeared without the appearance of new bands. This is in striking contrast to the corresponding chromatin experiments where the 370 bp fragment was converted within the first 30 min of incubation to the 332/342 bp fragments which then were stable for another 90 min of incubation (not shown). Also in the Hind III digestion experiments with the control samples (Fig. 3e, 5h-j) none of the three bands of the corresponding chromatin

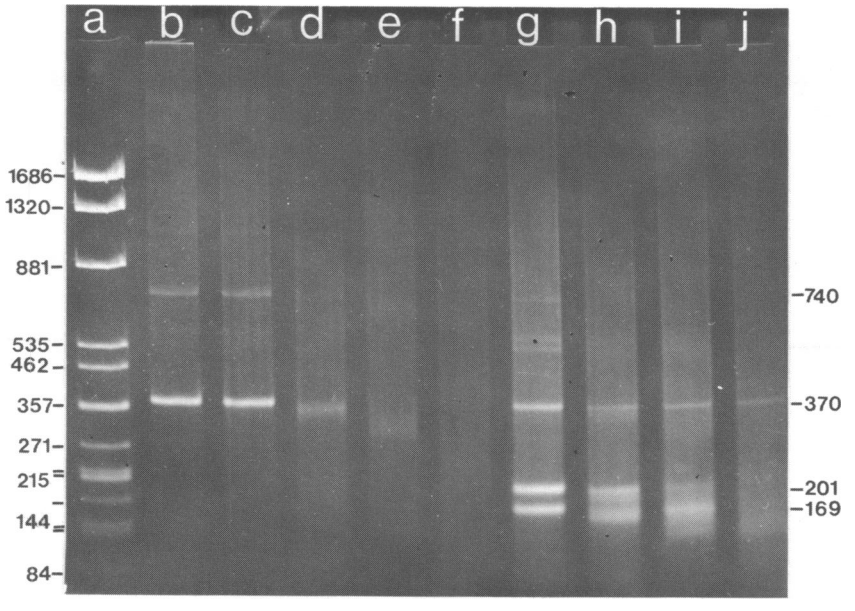
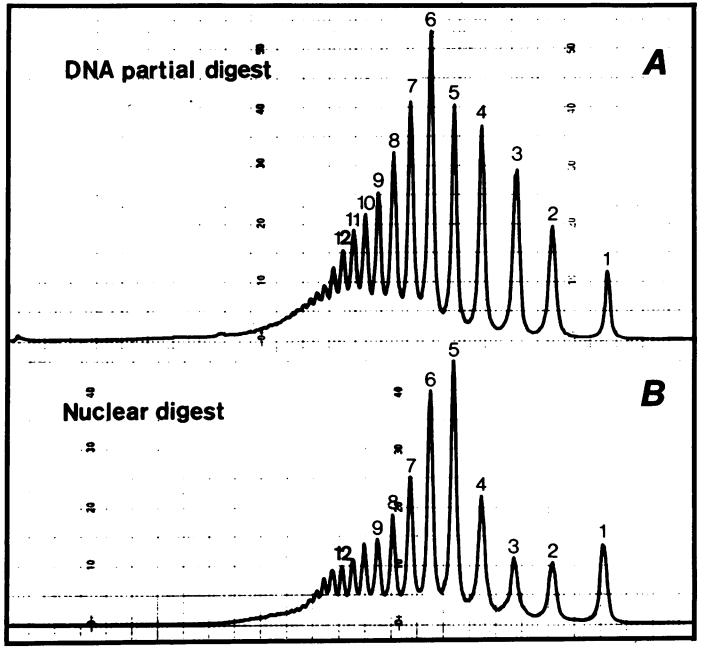


Figure 5. Absence of retardation sites for exonuclease III in the satellite I DNA sequence. Satellite I DNA was cleaved to completion with Hae III. DNA samples were digested to different degrees with exonuclease III (tracks c-f) and then with S1 nuclease to remove the exonuclease generated single stranded tails. Track a: a Hae III digest of λdv-1 plasmid DNA (23) as chain length markers. Track b: Hae III digest of satellite I DNA, no exonuclease/S1 treatment. Tracks c-f: 0' and 20' digests with 8 U/ml exonuclease III, 20' and 40' digests with 32 U/ml exonuclease III. Tracks g-j: aliquots of the digests c-f, respectively, were cleaved with Hind III. Electrophoresis was on a 2 % agarose gel and staining with ethidium bromide. Chain lengths are given in bp.

samples (Fig. 3c) appeared. Again a broadening and slow disappearance of the original bands was observed; the persistence of small amounts of fragments may be attributed to circular and linear aggregates which can be formed in exonuclease digests of satellite DNAs (24,25). In summary we conclude that the retardation sites in the exonuclease experiments with chromatin digests were due to specific nucleosome locations and not to peculiarities of the DNA sequence.

The rearrangement of nucleosomes is not the same on all of satellite I DNA. If the described nucleosome arrangement were valid for the whole satellite I DNA containing chromatin, all Hae III restriction sites would be located in linker regions and, with that, accessible to Hae III. A limit digest of nuclei should resemble closely a limit digest of DNA. By comparing tracks a and b of Fig. 2 it is evident that this is not the case. In the nuclear limit digest a considerable amount of higher multiples of the 370 bp monomer unit was present. Thus, in nuclei, some of the Hae III restriction sites are inaccessible and, therefore, the nucleosomes in part of the satellite containing chromatin must be in a different arrangement. This can be more clearly seen in Fig. 6 where Hae III digests of DNA and nuclei are compared. The fragment distribution obtained by DNA digestion is close to a normal distribution as shown by statistical analysis (Fig. 6C). In contrast, the analysis of the nuclear digest (Fig. 6B) indicates a non-random fragment distribution. At least two populations of fragments seem to be present corresponding to two different types of nucleosome arrangements.

The relative amount of satellite I DNA arranged in the way found for the 370 bp fragments and the amount arranged differently cannot be estimated exactly. The first type of arrangement occurs in at least 14 % of the satellite I DNA containing chromatin. This number is based on the amount of 370 bp fragment found in the nuclear digest (densitometry of track b of Fig. 2). If one corrects for preferential losses of short chain length DNA during transfer from gels to nitrocellulose, one arrives at a value of 34 %. We have indications from the analysis of sucrose gradient centrifugations of Hae III digests of nuclei (not shown) that the same type of arrangement is present on the dimer, trimer



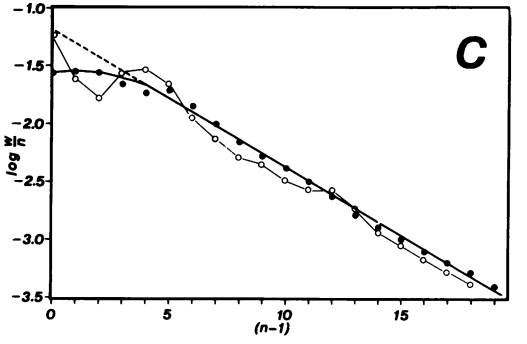


Figure 6. Distribution of Hae III cleavage sites in rat liver DNA and chromatin. A partial digest of rat DNA with Hae III (A) and DNA from a limit digest of rat liver nuclei with Hae III (B) were subjected to electrophoresis on a 0.35 % agarose slab gel. The DNA was then transferred to nitrocellulose (26) and hybridized with ^{32}P-labelled satellite I DNA. Autoradiograms were scanned (27) and densitograms are shown. (C) Quantitative analysis of the partial DNA digest (full symbols) and of the nuclear digest (open symbols). The weights of the bands (w), expressed as fractions of 1, were determined by planimetry and divided by the band number (n). The logarithm of the values were plotted as function of (n-1). Correction factors for losses of short chain length DNA during transfer from the gel to nitrocellulose filters were derived from the difference between the extrapolated (dashed) line and the measured (continuous) line. if a straight line is obtained in such a plot, the mode of cleavage is compatible with random cutting and the relative proportions of the bands agree with a normal distribution (28,29).

and perhaps also on the tetramer fragments, but not on the pentamer and higher multiples. This means that about half of the satellite I containing chromatin is organized in the way shown for the 370 bp Hae III fragment. The remainder is organized in a different, possibly also specific way.

DISCUSSION

Evidence was presented for a non-random arrangement of nucleosomes with respect to the sequence of satellite I DNA of rat liver. Hae III generated dinucleosomes from satellite I containing chromatin were studied in detail and the location of nucleosomes in three defined positions could be established. Part of the satellite DNA containing chromatin, however, is organized in another, still unknown fashion. The different arrangements of nucleosomes may occur on different arrays of the satellite DNA, for example on different chromosomes or separated arrays on the same chromosome; alternatively, they may occur on the same array of the satellite DNA chain, giving rise to "transitions" between the different nucleosome arrangements. The experiments described in this paper do not give any hint as to which of the two possibilities is realized in satellite I containing chromatin. Experiments on the kinetics of exonuclease III digestion of DNA from a limit Hae III digest of rat liver chromatin (not shown) indicate the existence of transitions between different nucleosome arrangements within the same array, but do not prove them.

The structural reasons for the specificity in the nucleosome arrangement on rat satellite I DNA are unclear at present. One may consider a preference of binding of the histone octamer or of some non-histone proteins for certain DNA sequences. Alternatively one may invoke geometric arguments: a preference for the occurrence of certain dinucleotides at a 10.5 bp average spacing, as was found recently by a statistical analysis of published DNA sequences may provide a mechanism for specific nucleosome arrangements on the DNA chain (30). One may speculate that certain aspects which lead to the non-random arrangement of nucleosomes on satellite I DNA of the rat will hold for other satellite DNAs and possibly also for non-repetitive DNA.

ACKNOWLEDGMENTS

We are grateful to Irene Brenner for expert technical assistance. Akira Omori is a fellow of Alexander von Humboldt Foundation and is also indebted to Mitsubishi-Kasei Institute of Life Sciences. The work was supported by Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

REFERENCES

1. Polisky, B. and McCarthy, B. (1975) Proc. Natl. Acad. Sci. US 72, 2895-2899.
2. Cremisi, Ch., Pignatti, P.F. and Yaniv, M. (1976) Biochem. Biophys. Res. Commun. 73, 548-554.
3. Ponder, B.A.J. and Crawford, L.V. (1977) Cell 11, 35-49.
4. Waldeck, W., Föhring, B., Chowdhury, K., Gruss, P. and Sauer, G. (1978) Proc. Natl. Acad. Sci. US 75, 5964-5968.
5. Scott, W.A. and Wigmore, D.J. (1978) Cell 15, 1511-1518.
6. Nedospasov, S.A. and Georgiev, G.P. (1980) Biochem. Biophys. Res. Commun. 92, 532-539.
7. Wasylyk, B., Oudet, P. and Chambon, P. (1979) Nucl. Acids Res. 7, 705-713.
8. Prunell, A. and Kornberg, R.D. (1977) Cold Spring Harbor Symp. Quant. Biol. 42, 103-108.
9. Baer, B.W. and Kornberg, R.D. (1979) J. Biol. Chem. 254, 9678-9681.
10. Wittig, B. and Wittig, S. (1979) Cell 18, 1173-1183.
11. Gottesfeld, J.M. and Bloomer, L.S. (1980) Cell in press.
12. Wu, C. (1980) Nature 286, 854-860.
13. Pfeiffer, W. and Zachau, H.G. (1980) Nucl. Acids Res. in press.
14. Musich, P.R., Maio, J.J. and Brown, F.L. (1977) J. Mol. Biol. 117, 657-677.
15. Gottesfeld, J.M. and Melton, D.A. (1978) Nature 273, 317-319.
16. Singer, D.S. (1979) J. Biol. Chem. 254, 5506-5514.
17. Fittler, F. and Zachau, H.G. (1979) Nucl. Acids Res. 7, 1-13.
18. Omori, A., Igo-Kemenes, T. and Zachau, H.G. (1980) Nucl. Acids Res. 8, 5363-5375.
19. Pech, M., Igo-Kemenes, T. and Zachau, H.G. (1979) Nucl. Acids Res. 7, 417-432.
20. Igo-Kemenes, T., Greil, W. and Zachau, H.G. (1977) Nucl. Acids Res. 4, 3387-3400.
21. Vogt, V.M. (1973) Eur. J. Biochem. 33, 192-200.
22. Riley, D. and Weintraub, H. (1978) Cell 13, 281-293.
23. Streeck, R.E. and Zachau, H.G. (1978) Eur. J. Biochem. 89, 267-279.
24. Thomas, C.A. Jr., Pyeritz, R.E., Wilson, D.A., Dancis, B.M., Lee, C.S., Bick, M.D., Huang, H.L. and Zimm, B.H. (1973) Cold Spring Harbor Symp. Quant. Biol. 38, 353-370.
25. Schachat, F.H. and Hogness, D.S. (1973) Cold Spring Harbor Symp. Quant. Biol. 38, 371-381.
26. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.

27. Hörz, W. and Zachau, H.G. (1980) submitted.
28. Southern, E.M. (1975) J. Mol. Biol. 94, 51-69.
29. Altenburger, W., Hörz, W. and Zachau, H.G. (1977) Eur. J. Biochem. 73, 393-400.
30. Trifonov, E.N. (1980) Nucl. Acids Res. 8, 4041-4053.