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Nucleosome arrangement in α-satellite chromatin of African green monkey cells

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ABSTRACT

By analyzing the accessibility of restriction endonuclease sites in African green monkey α-satellite chromatin, we demonstrate the absence of a unique phase relationship between nucleosomes and α-satellite DNA. The data indicate a minimum of three different positions for nucleosome cores relative to the α-satellite sequence and suggest a random distribution in at least some regions. In addition, while we confirm published reports that staphylococcal nuclease cuts the α-satellite sequence in chromatin at a highly preferred site, two-dimensional gel electrophoresis of nuclear digests demonstrates that this site is preferentially cut by staphylococcal nuclease even when it is within the nucleosome core. These data indicate that staphylococcal nuclease is not useful for determining nucleosome positions on α-satellite DNA, and perhaps on other specific DNA sequences as well.

INTRODUCTION

The relation, if any, between nucleosome position and DNA sequence, i.e., phasing, may be an important determinant of chromatin structure and function (for review see 1). Many studies of phasing have utilized the highly repetitive non-transcribed α-satellite DNA of the African green monkey. This satellite DNA consists of a 172 bp tandemly repeated family of sequences representing 10-20% of the genome (2, 3), for which a consensus sequence has been published (2). Conflicting reports have suggested either the presence (4, 5) or the absence (3, 6) of a unique phase relationship. Arguments for phasing are based on digestion of nuclei with staphylococcal nuclease (4, 5); however, because of the sequence specificity of this enzyme (7-9) this interpretation has been challenged (6). Two recent reports suggest complex, non-random relationships between nucleosome core position and the

α-satellite sequence (10, 11). One of these studies concludes that there are a number of preferred positions of nucleosome cores on α-satellite DNA (11); however, there are no data on the relative positions of adjacent cores.

In this report, we utilize a fortuitous distribution of Dde I and Mbo II cleavage sites within the α-satellite repeat to demonstrate, independently of staphylococcal nuclease, that there is not a unique phase relation between nucleosome and α-satellite repeats. Furthermore, we demonstrate that the apparent phasing seen in experiments using staphylococcal nuclease (4, 5) results, at least in part, from cleavage by this enzyme of a preferred site in the α-satellite sequence, even if this site is within the nucleosome core. Thus, staphylococcal nuclease may be of limited value for mapping the positions of nucleosomes with respect to specific DNA sequences.

MATERIALS AND METHODS

Cell Culture.

The African green monkey kidney cell line BS-C-1 (ATCC CCL 26) was maintained in culture as suggested by the supplier. Cells were either split 1:4 or refed at intervals of no more than 14 days and used for nuclear isolation at least 7 days after being subcultured.

Isolation of Nuclei and DNA.

Nuclei were isolated by previously described methods (12), except 1 mM $MgCl_2$ replaced 1 mM $CaCl_2$. Genomic DNA was isolated by the method of Davis, et al. (13). To obtain consistent digestion of DNA with restriction enzymes, it was necessary to reprecipitate the DNA two times with ethanol.

Restriction Endonuclease Digestion of Nuclei and DNA.

Restriction endonuclease digestions were carried out in 6 mM Tris (pH 7.6), 6 mM $MgCl_2$ and 75 mM NaCl at a DNA concentration of 100 µg/ml for 2 hours at 37°C with 5 U enzyme per µg for purified DNA and 10 U/µg DNA for nuclei. All restriction enzymes were from Bethesda Research Laboratories. Digestions were stopped by addition of Na_2EDTA to 10 mM and SDS to 0.5%, and chilling on ice. DNA was then isolated by digestion with proteinase K (300 µg/ml) at 55°C for one hour, extraction with phenol, extraction with chloroform:isoamyl

alcohol (24:1), and then ethanol precipitation. Samples were dissolved in 10 mM Tris (pH 8)-0.25 mM EDTA for electrophoresis or re-restriction. Re-restriction was under the conditions described above, with the addition of 500 µg/ml bovine serum albumin (BSA) in case residual proteinase K was present.

Staphylococcal Nuclease Digestion of Nuclei.

Nuclei, in 0.25 M sucrose, 10 mM Tris (pH 8), and 1 mM $MgCl_2$ were adjusted to final concentrations of 100 µg DNA/ml, 80 mM NaCl, and 0.25 mM $CaCl_2$. Staphylococcal nuclease (Worthington Co.) was added and incubation was at 0° or 37°C for the times indicated. Digestion was stopped by addition of EDTA to 10 mM and chilling on ice. DNA was isolated from staphylococcal nuclease nuclear digests as from restriction digests.

Mapping of Staphylococcal Nuclease Digestion Sites in α-Satellite Chromatin.

The cleavage sites were mapped relative to the Hind III site by hybridization . Isolated DNA was digested with Hind III, re-isolated, subjected to 5% polyacrylamide gel electrophoresis, transferred to a nitrocellulose filter and hybridized to pCa85 (see below).

Polyacrylamide Gel Electrophoresis and Electrophoretic Transfer to Nitrocellulose.

Electrophoresis was on 150 x 100 x 3 mm 5% polyacrylamide (29:1, acrylamide:bis) gels in TBE buffer (14) at 100 V for 90 min. Gels were stained with ethidium bromide (2 µg/ml) and photographed under UV transillumination on Polaroid type 55 film with an orange filter. For electrophoretic transfer of DNA from polyacrylamide gels to nitrocellulose filters (15), gels were shaken at room temperature for 20 min each in 4x, 2x, and finally 3 changes of 1x transfer buffer, which was 20 mM phosphate-5 mM citrate (pH 3). Transfer was at 28 volts and 2 amp for 2 hr.

Hybridization to Nitrocellulose Filters.

After transfer, nitrocellulose filters were dried at 55°C overnight and pretreated with 3XSSC-1X Denhardt's solution at 55°C for at least 2 hr. The probe, plasmid pCa85 (generously supplied by Drs. R. Thayer and M. Singer), which contains an

α-satellite monomer inserted into the Hind III site of pBR322 (16), was isolated and nick-translated (Amersham Nick Translation Kit) with [α-^{32}P]dCTP. Hybridization was in 3XSSC-1X Denhardt's solution at 55°C for 48-60 hr with 1-2 x 10^6 cpm/ml in 8-10 ml total volume. Following hybridization, the filter was washed in 3 changes of 3XSSC-0.1% SDS at 55°C, dried at 80°C for 2 hr., and autoradiographed for 18 hr. at -70°C with an intensifying screen, using Kodak X-O-Mat film.

End Labeling.

DNA was end-labeled by the T4 kinase exchange reaction by mixing DNA eluted from a polyacrylamide gel with 50 μCi [γ-^{32}P]ATP in 50 mM imidazole (pH 6.6), 12 mM $MgCl_2$, 10 mM dithiothreitol, 300 μM ADP and 12 μM ATP, and incubating with 10 units of T4 kinase at 37°C for 20 min in a total volume of 25 μl. DNA was purified by three cycles of ethanol precipitation.

Two-Dimensional Gel Electrophoresis and Hybridization.

Nuclei were digested with staphylococcal nuclease (0.01 U/μg DNA, 37°C) for various times, or with Dde I (10 U/μg DNA, 37°C) for 2 hr. Ice-cold EDTA was added to 1 mM and the samples were chilled on ice and centrifuged at 12,000 g for 2.5 min. The pellets were resuspended in 10 mM Tris (pH 8), 0.25 mM EDTA at 500 μg DNA/ml. After 30 min on ice, 20 μl aliquots were made 10% in glycerol with bromophenol blue as marker dye. The 2-D gel system was adapted from Levinger et al. (17). The first dimension was a 20 x 14 x 0.4 cm 0.7% agarose gel in 3 mM EDTA, 2 mM sodium acetate (pH 5.5) run in a submersible apparatus at 4°C for 3 hr at 175 V with buffer recirculation. After ethidium bromide staining, the staphylococcal nuclease digest with the distribution of DNA closest to that of the Dde I limit digest was selected and these two lanes were cut out and placed in 1% SDS, 1 mM EDTA, 2 mM Tris (pH 8), 0.01% bromophenol blue for 1 hr at 37°C. The two lanes were then cast 15 cm apart in the same 30 x 18 x 0.5 cm 1.2% agarose gel in 0.1% SDS, 40 mM Tris (pH 7.6), 5 mM sodium acetate, 1 mM EDTA and electrophoresed for 18 hr at 40 V. The DNA from each half of the gel was transferred to a separate 15 x 15 cm nitrocellulose filter by standard procedures (18), prehybri-

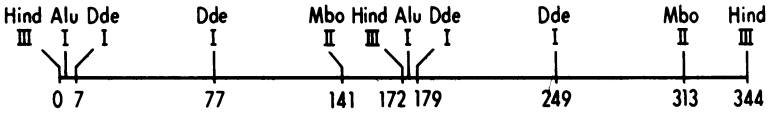

Figure 1. Partial restriction endonuclease map of the 172 bp α-satellite sequence of African green monkey (two tandem repeats are shown).

dized in 3XSSC-1X Denhardt's solution at 55°C for at least 5 hr, and hybridized to 1 x 10^7 cpm of nick-translated pCa85 in 15 ml fresh 3XSSC-1X Denhardt's solution for 48 hr at 55°C. The filters were washed, dried and autoradiographed as described above.

RESULTS

Restriction Endonuclease Digests of African Green Monkey Kidney (AGMK) DNA and Nuclei.

A partial restriction map of α-satellite DNA as predicted from the consensus sequence (2) is shown in figure 1. The presence of the computer predicted sites in most repeats has been verified by digesting AGMK DNA with Alu I, Hind III, Mbo II, and Dde I (Fig. 2A, odd-numbered lanes). In contrast, when nuclei are digested, even with high amounts of these restriction enzymes, there is a lesser degree of digestion (Fig. 2A, even-numbered lanes), suggesting that not all sites are accessible in chromatin. For both nuclei and DNA, these are limit digests, since addition of more enzyme at 2 hr does not change the digestion pattern (data not shown). While there is some endogenous exonuclease "trimming" activity (Fig. 2A, lanes 2, 4, 6, and 8), controls (lane 9 and 10) rule out significant endogenous endonuclease activity, confirming previous findings in AGMK cells (4).

Although most α-satellite DNA repeats contain a Hind III site, virtually no Hind III digestion occurs in nuclei (Fig. 2A, lanes 3 and 4). Since some Dde I sites only seven base pairs away are accessible and must be outside of the core (see below), failure of Hind III to cleave α-satellite chromatin is not the result of protection of all Hind III sites by core proteins. In addition, Alu I, which recognizes the inner

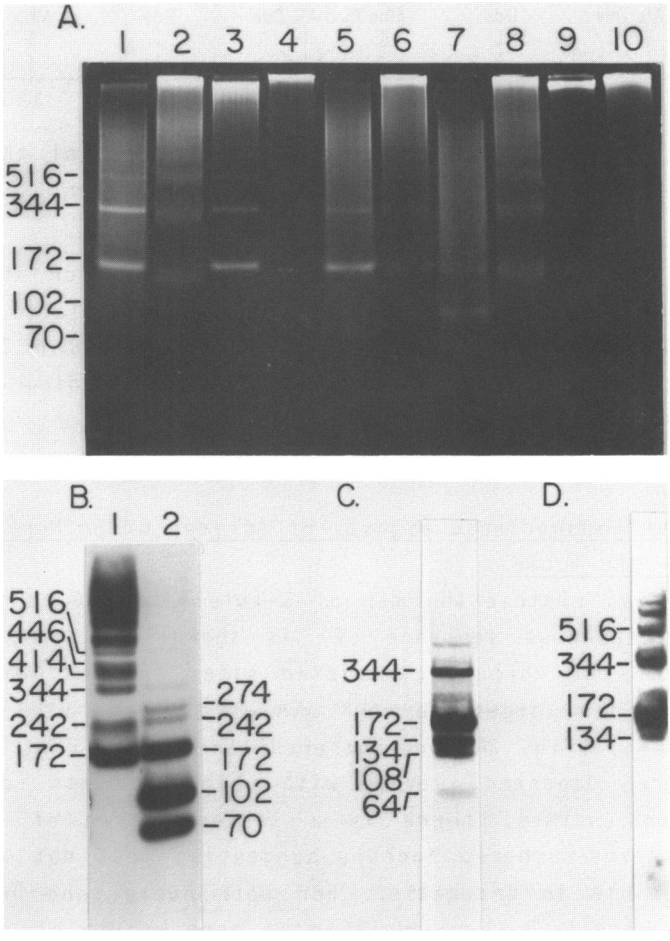

Figure 2. Restriction endonuclease digestion of nuclei and DNA. A. DNA (odd-numbered lanes) or nuclei (even-numbered lanes) were incubated with the indicated restriction enzyme; the DNA was isolated and electrophoresed on 5% acrylamide gels. The gel was photographed after ethidium bromide staining. Alu I (lanes 1,2); Hind III (lanes 3,4); Mbo II (lanes 5,6); Dde I (lanes 7,8); and no enzyme (lanes 9,10).
B. Nuclei (lane 1) or DNA (lane 2) were digested with Dde I. DNA was then isolated, subjected to electrophoresis on a 5% polyacrylamide gel, transferred to a nitrocellulose filter and hybridized to nick-translated pCa85.
C. Mapping of Dde I digestion sites in α-satellite chromatin. Nuclei were digested with Dde I; DNA was then isolated and digested with Mbo II. The DNA was then re-isolated and analyzed as in B.
D. Digestion of α-satellite chromatin with both Dde I and Mbo II. Nuclei were incubated with 10U of each enzyme per μg DNA for 6 hr. DNA was then analyzed as in B.

tetramer of the Hind III site, also cuts α-satellite chromatin (Fig. 2A, lane 2), confirming the above interpretation. Bacteriophage λ DNA mixed with the nuclei is digested by Hind III (data not shown), demonstrating that the enzyme is active under these reaction conditions. Lack of digestion of α-satellite chromatin by Hind III has been used to support arguments for phasing (4,10), but our data indicate that lack of Hind III cutting results from the inability of this enzyme to cut in chromatin, not from phasing.

Of particular interest is the analysis of the products of digestion of nuclei with Dde I since the two repeating Dde I sites are 70 and 102 bp apart. Thus, unless the distance between cores is longer than 70 bp, which is unlikely (e.g., 1), when one Dde I site is in a linker region the adjacent Dde I sites must be within cores. In contrast to Dde I digestion of DNA, Dde I digestion of nuclei generates no 70 or 102 bp fragments as analyzed either by ethidium staining (Fig. 2A, lanes 7 and 8) or by more sensitive hybridization methods (Fig. 2B, lanes 1 and 2). This observation is in accord with expectations that core proteins protect DNA from digestion by Dde I. The absence of 70 and 102 bp fragments in the Dde I digest of nuclei also argues against significant nucleosome sliding under the digestion conditions employed, and is consistent with the absence of linkers longer than 70 bp in α-satellite chromatin.

Nucleosome Arrangement in α-Satellite Chromatin.

The pattern of α-satellite DNA fragments generated by digestion of nuclei with Dde I (Fig. 2B) also supports the presence of a non-phased component of nucleosomes in α-satellite chromatin. If nucleosome core proteins are phased relative to the α-satellite DNA, implying that both the α-satellite and the nucleosome repeat lengths are 172 bp, then digestion of chromatin should produce only 172 bp fragments (and multiples thereof where sites are degenerate) or no digestion (Fig. 3A). Even if different positions for core proteins on the α-satellite sequence were found in different regions of α-satellite DNA, within each stretch of tandem repeats only a ladder of 172 bp multimers would be generated.

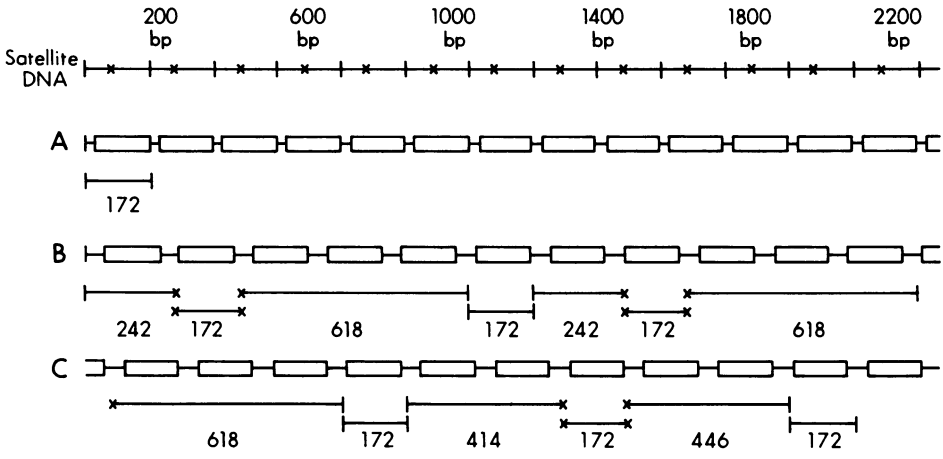

Figure 3. Distribution of restriction sites in α-satellite chromatin. The two Dde I sites are indicated superimposed on a repeating nucleosome structure, with the core represented by the rectangle (drawn to scale with a length of 146 bp), and linker equal to 26 bp. (A) or 50 bp (B,C). In A, note that a shift in the phase could result in digestion of the other Dde I site, or of neither site. In B and C, the difference between nucleosome and α-satellite repeat lengths results in variable restriction site accessibility. The bottom section indicates fragment lengths that would be generated from each segment of α-satellite chromatin.

Yet digestion of nuclei with Dde I produces other fragments in a specific pattern, e.g. 242 and 414 bp, along with 172 and 344 bp fragments. These fragments can only arise from regions in which the nucleosome and α-satellite repeat lengths differ, i.e. regions which are not simply phased. (See next section and Fig. 3 for discussion of the presence or absence of bands corresponding to specific fragments that could potentially be generated.)

In a separate approach, we mapped the Dde I digestion sites in α-satellite chromatin by isolating DNA from Dde I-digested nuclei and redigesting the DNA with Mbo II (Fig. 2C). A unique phasing of nucleosomes with respect to the α-satellite sequence would predict that only one of the two Dde I sites in a 172 bp repeat would ever be accessible in nuclei, while the other site would always be protected by core proteins (see Fig. 3A). In such a case, analysis of the restriction map in Fig. 1 indicates

that Mbo II digestion of DNA isolated from Dde I digested nuclei would yield a pair of fragments either 108 and 64 bp long (if the Dde I site at 77 and 249 were accessible), or 134 and 38 bp long (if the Dde I site at 7 and 179 were accessible). In fact, both pairs of bands are produced (Fig. 2C). [The 64 bp band is faint and does not reproduce well. The 38 bp band is not seen since hybridization to fragments of this length is inefficient (15)]. These data confirm that, in a given α-satellite sequence, either the site at 7 or the site at 77 (see Fig. 1) may be accessible to Dde I. Since, as demonstrated above (Fig. 2B, lane 1), Dde I does not cleave significantly within core, the accessible sites must occur in linker, and, therefore, there must be a minimum of two positions in which nucleosome core proteins bind to α-satellite DNA in chromatin. Digestion of nuclei with Mbo II (Fig. 2A, lane 6) also generates a ladder of 172 bp multimers. Much high molecular weight material remains, suggesting that, as with Dde I, core proteins protect DNA from digestion with Mbo II. Furthermore, if Mbo II did cut within core, then double digestion of nuclei with both Mbo II and Dde I would yield a pattern of fragments similar to that of Fig. 2C, with 134 bp as well as 108 and 64 bp fragments visualized. Such a double digest, even when allowed to proceed for 6 hr. (Fig. 2D), produced no 108 and 64 bp fragments. The presence of some 134 bp material may reflect cutting near each end of a core, especially since the Mbo II recognition site is centered 11 bp further away from the Dde I site, so that the distance between the recognition sites for this Dde I-Mbo II fragment approaches the usual length of core DNA. Mbo II, therefore, probably does not cut significantly within core particles. Thus, the Mbo II site must appear within linker at some points in α-satellite chromatin where both of the adjacent Dde I sites are in core. This result indicates that there are a minimum of three arrangements of nucleosomes with respect to α-satellite DNA.

Model of Random Distribution of Nucleosomes.

Using a mathematical model, we sought to determine if the pattern of DNA fragments generated by Dde I digestion of α-satellite chromatin was consistent with a random distribution

of nucleosome cores. The simplest model is one in which core and linker lengths are fixed, there are no preferential positions relative to the DNA, the probability of cutting at a restriction site is 1 when in linker and 0 within core, and the probability of the predicted 4 base Dde I recognition site being present in any particular α-satellite repeat is estimated as $(0.98)^4$(2). Given that there is a restriction enzyme cleavage in a linker, the model calculates P_L, the probability that a fragment of length L will be generated using the equation $P_L = (P_l)\ (P_u)\ (0.98)^4$, where P_l is the probability that the site corresponding to fragment length L is within linker, and P_u is the probability that all closer restriction sites are uncleaved.

This model does not account for variable linker lengths, preferred core positions (11) or clustering of restriction sites. If one assumes a fixed core length of 146 bp of DNA, this simple random model predicts that if the internucleosomal linker accessible to Dde I is greater than 48 bp, then the 242 bp fragment can be generated as a nucleosome core plus two adjacent linkers, while a 274 bp fragment is forbidden unless the linker is greater than 64 bp. Presence of the 242 bp band and absence of the 274 bp fragment, predicted by the random model (Fig. 3), is seen experimentally (Fig. 2B, lane 1). Similarly, the 414 bp fragment would be seen with linker lengths greater than 42 bp, while a 446 bp fragment would only be seen with linkers longer than 52 bp. Thus, a nucleosome repeat length of about 200 bp randomly positioned with respect to the 172 bp α-satellite repeat (see Fig. 3) would generate a pattern of DNA fragments similar to that observed here (Fig. 2B, lane 1), so that our data are consistent with the hypothesis that a substantial portion of α-satellite chromatin has randomly placed nucleosomes.

Mapping of Staphylococcal Nuclease Digestion Sites in Nuclei.

It has been suggested that a unique phasing of nucleosomes in α-satellite chromatin can be detected only under certain specific conditions (4). To confirm that our finding of multiple nucleosome positions in α-satellite chromatin is not an artifact of our nuclear isolation and digestion conditions,

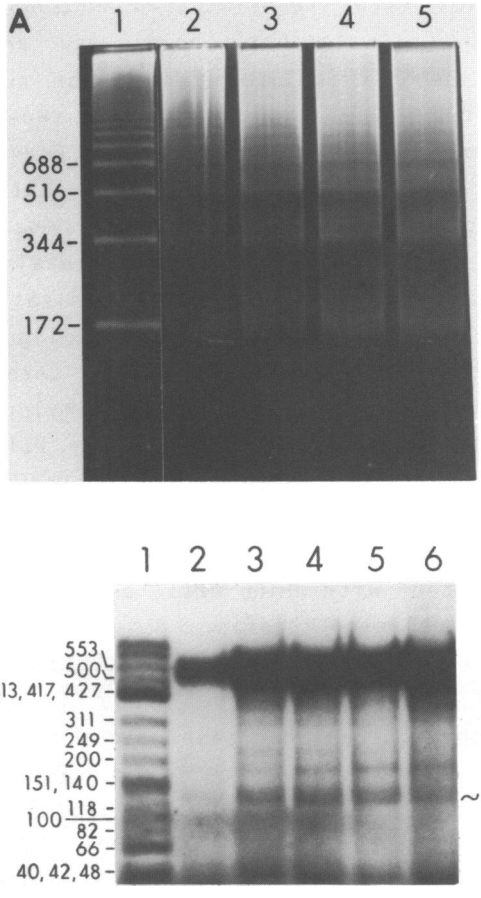

Figure 4. A. Time course of staphylococcal nuclease digestion of nuclei. Nuclei were digested with staphylococcal nuclease (1 U/µg DNA) for varying times on ice. DNA was isolated and subjected to 5% polyacrylamide gel electrophoresis. The gel was stained with ethidium bromide and photographed. For lanes 2-5, digestion was for 8, 12, 16, and 20 min, respectively. Lane 1 contains AGMK DNA digested with Hind III (5 U/µg, 37°C, 2 hr).
B. Mapping of staphylococcal nuclease cleavage sites in α-satellite chromatin by end-labeling. Trimer DNA in the gel of figure 4A was eluted, end-labeled, and the DNA from lanes 2-5 digested with Hind III. The DNA was reisolated and electrophoresed. The gel was dried and autoradiographed. Lane 1 contains ϕX174 DNA digested with Hinf I and end-labeled. Lane 2 contains 516 bp trimer DNA from AGMK DNA digested with Hind III. In lanes 3 to 6, corresponding to lanes 2-5 in Figure 4A, digestion with staphylococcal nuclease was for 8, 12, 16, and 20 min, respectively.

we digested nuclei with staphylococcal nuclease at either 4° (Fig. 4A) or 37°C (data not shown) and analyzed by gel electrophoresis the DNA isolated from these nuclei. The DNA fragments exhibited a 172 bp repeat ladder superimposed on the typical nucleosome oligomeric pattern, as previously reported (4-6). Transfer of the DNA fragments to nitrocellulose and hybridization to the α-satellite probe (data not shown) confirmed that the 172 bp multimers contained α-satellite sequences (4, 6). A minor amount of hybridization detected in the regions of bulk chromatin nucleosome oligomers may arise from boundaries between α-satellite and bulk chromatin.

We mapped the position of the staphylococcal nuclease cutting sites with respect to the Hind III site of the α-satellite sequence essentially by the procedure of Musich et al. (5). Trimer DNA from staphylococcal nuclease digestion of AGMK nuclei at 4°C was eluted from the acrylamide gel, endlabeled, digested with Hind III and analyzed again by polyacrylamide gel electrophoresis. Autoradiograms demonstrate (Fig. 4B) a distinct band at about 125-130 bp, similar to that found by Musich et al. (5), that persists with increasing extents of staphylococcal nuclease digestion. We obtained similar results with dimer DNA. Thus, under our reaction conditions we see the preferential staphyloccal nuclease cutting site previously reported (4-6).

Staphylococcal Nuclease Cuts Within Nucleosome Cores.

One hypothesis which reconciles the lack of a single, unique nucleosome phasing, as determined by digestion with restriction endonucleases (Fig. 2) with the observed specific cleavage by staphylococcal nuclease, is that staphylococcal nuclease digests one highly preferred site in α-satellite chromatin even if that site is within the nucleosome core. In such a case, α-satellite oligonucleosomes should contain double stranded breaks in the DNA at integral multiples of 172 bp, held together by nucleosome core protein interactions. To test this hypothesis, we digested nuclei with staphylococcal nuclease and used a two-dimensional gel system (17) to separate nucleoprotein in the first dimension and DNA in the second dimension. Similar experiments by others (10) have demon-

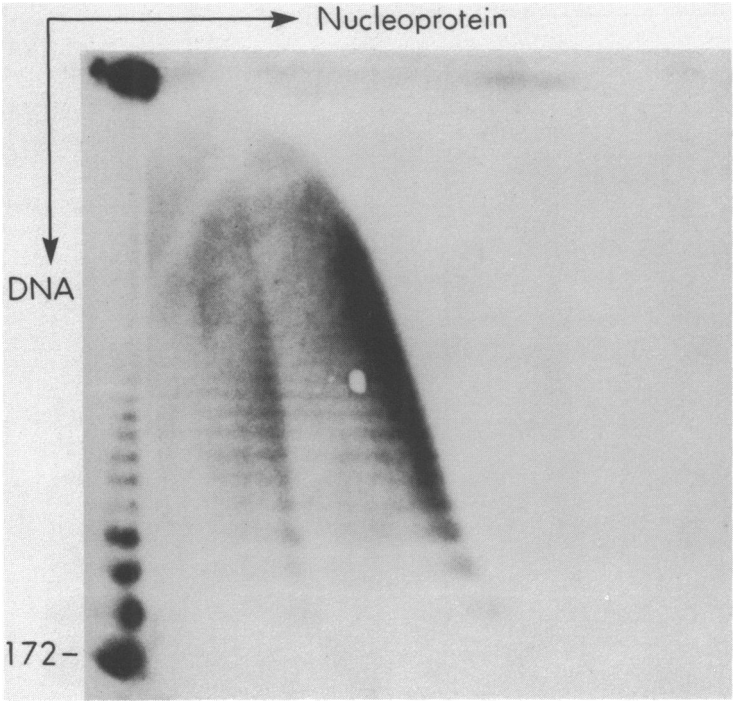

Figure 5. Hybridization of α-satellite DNA to a two-dimensional gel pattern of nuclei digested with staphylococcal nuclease. Nuclei digested with staphylococcal nuclease (0.01U/μg DNA, 5 min at 37°C) were electrophoresed in the nucleoprotein dimension in 0.7% agarose at low ionic strength, and then in the DNA dimension in 1.2% agarose in the presence of SDS, blotted onto nitrocellulose, hybridized to pCa85 and autoradiographed. Markers, somewhat distorted by being at the edge of the gel and blot, are a partial Hind III digest of AGMK DNA.

strated the absence of such "hidden" cuts in EcoRI-digested α-satellite chromatin. In our experiments, visualization of the DNA by ethidium bromide staining showed only the expected diagonal band for bulk DNA. The DNA was then transferred to a nitrocellulose filter and hybridized to the α-satellite probe. Autoradiograms (Fig. 5) clearly demonstrate that nucleoprotein oligomers produced by staphylococcal nuclease digestion of α-satellite chromatin contain not only DNA of the length appropriate for the oligomer size (dense diagonal band), but also DNA cut into smaller multiples of 172 bp. (The second

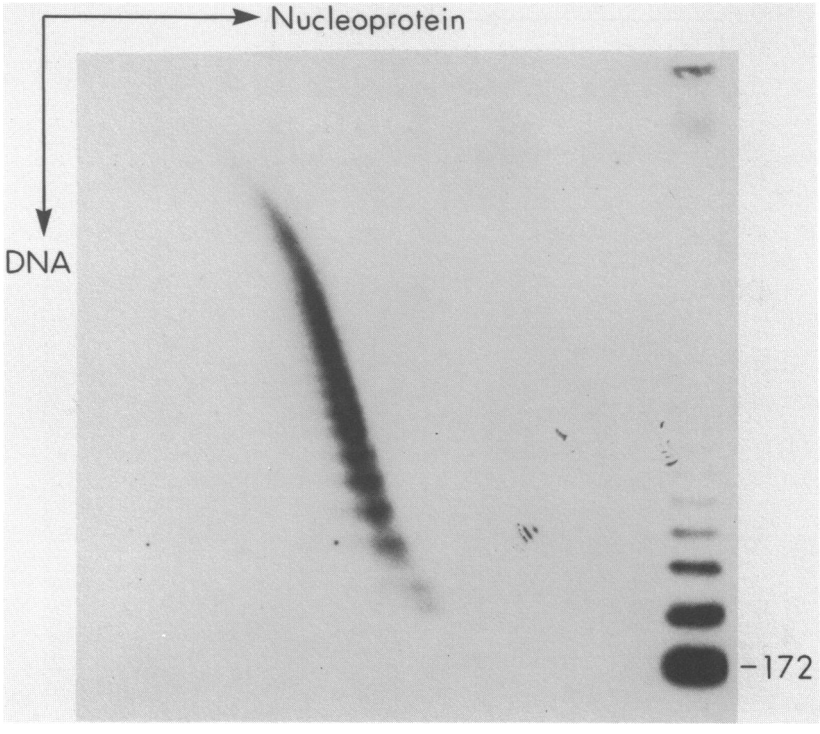

Figure 6. Hybridization of α-satellite DNA to a 2-D gel pattern of nuclei digested with Dde I. The same procedure as for Figure 5 was followed, except nuclei were digested with Dde I (10 U/μg DNA,37 °C, 2 hr) instead of staphylococcal nuclease. Markers are AGMK DNA digested with Hind III.

less intense diagonal band was not seen in all experiments and its source is unclear, but also see Ref. 10, Fig. 4C.) For comparison, we used the 2-D gel system to analyze oligonucleosomes from nuclei digested with Dde I instead of staphylococcal nuclease (Fig. 6). Virtually all of the α-satellite DNA from the Dde I digest falls on the diagonal predicted if each oligonucleosome has no internal double strand breaks, confirming that Dde I does not cut significantly within the nucleosome core. The Dde I results also control for artifactual production of regularly spaced breaks in the α-satellite DNA during the procedure. Amount of sample DNA, hybridization conditions and exposure times were the same for Figs. 5 and 6.

DISCUSSION

Nucleosome Arrangement in α-Satellite Chromatin as Determined by Restriction Endonuclease Digestion.

The accessibility in individual repeats of α-satellite chromatin of only one of three restriction endonuclease sites, the Dde I site at 7, the Dde I site at 77 or the Mbo II site at 141 rules out a unique phasing of nucleosomes in α-satellite chromatin and indicates that nucleosome cores may assume at least three, and probably more, positions with respect to the α-satellite sequence. The specific pattern of α-satellite DNA fragments generated by the digestion of nuclei with Dde I is consistent with the presence of regions with random distribution of nucleosomes on α-satellite DNA. Within such a random distribution, core proteins might have local sequence preferences so that, while not directly comparable, our data are also consistent with the demonstration of multiple specific positions for core proteins on α-satellite DNA (11).

Wu, *et al*. recently analyzed the small subset of α-satellite chromatin solubilized by digestion with EcoRI by redigestion with a second restriction endonuclease. Their interpretation of the data indicates a preferred arrangement for cores on these regions of α-satellite DNA. While we have no evidence bearing directly on this specific chromatin fraction, we have shown (Fig. 2A) that lack of digestion of chromatin with Hind III is not necessarily due to protection of sites by core proteins. Further, while their finding that all Hae III sites are accessible in this fraction of α-satellite chromatin is consistent with specific core protein positions, it is also possible that Hae III, like staphylococcal nuclease, can digest within cores. By conceptualizing a repeating pattern of nucleosomes of a fixed length other than 172 bp overlying the tandem 172 bp α-satellite DNA repeats (see Fig. 3), as in our model, one sees that, as with the interference pattern of two waves of different wavelength, a given site in the α-satellite repeat would periodically move out of phase and then back into phase with linker over a number of repeats. Allowing for some variability in linker lengths, digestion of α-satellite chromatin containing a random distribution of

nucleosomes by a restriction endonuclease that cuts once per 172 bp repeat would give rise to a broad spectrum of fragment lengths in which all potential internal cleavage sites would be protected by core proteins, as was found (10). Thus, while we cannot rule out a preferred arrangement of nucleosomes in EcoRI solubilized α-satellite chromatin, a random distribution remains a possibility.

Staphylococcal Nuclease Cuts Within Nucleosome Cores.

We have confirmed previous data (4-6) indicating that staphylococcal nuclease cuts the α-satellite sequence in chromatin at a highly preferred site; however, our further analysis, using two-dimensional gel electrophoresis, indicates that staphylococcal nuclease cuts at this site whether it is in core or in linker. Thus, mapping staphylococcal nuclease cleavage sites is not useful for determining nucleosome position in α-satellite chromatin.

The finding that staphylococcal nuclease digests a specific DNA sequence even when that sequence is located in the nucleosome core has broad implications for the study of chromatin structure of specific genes. This enzyme has been widely used (4, 19-21) to map the position of nucleosomes on specific DNA sequences. Our data indicate, however, that merely mapping staphylococcal nuclease sensitive sites is not sufficient to define linker positions. Since staphylococcal nuclease has DNA sequence as well as chromatin structural preferences, the kinetics of digestion will depend on the interplay of these two factors. For bulk chromatin, linker preference generally far outweighs sequence preference. In the case of α-satellite chromatin, however, with strong sequence preference, digestion appears to proceed initially at preferred sites that lie within linker, then at preferred sites within cores, and then at other sites within linkers. Furthermore, in addition to the sequence specificity of the enzyme on free DNA, chromatin structure itself can alter the relative sequence preference of the enzyme. Thus, staphylococcal nuclease digestion of free DNA may not always be an adequate control for sequence specificity when chromatin digestion is examined. It appears that the effect of chromatin structure on staphyloccal

nuclease sensitivity is considerably more complex than was previously believed.

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