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Nucleosome spacing in rat liver chromatin. A study with exonuclease III

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ABSTRACT

Exonuclease III was used to uniformly trim DNA ends of micrococcal nuclease-prepared chromatin fragments down to the first major impediment encountered by the enzyme, which arises from the interaction of H1 with the nucleosome. This trimming, when performed on nucleosome dimers, allowed one to quantitatively determine the center-to-center distance of nucleosomes. This distance, of mean 198 base pairs, was found to essentially vary between about 180 and 215 base pairs, with extremes of 165 and 230 base pairs. Trimming of trimers further revealed that the overall arrangement of nucleosome center-to-center distances along the chromatin fiber is that expected on a statistical basis.

INTRODUCTION

Specific positions of nucleosomes on particular sequences may regulate the pattern of recognition of these sequences by regulatory proteins or other molecules. This prospect has recently stimulated a wide effort to find out correlations between gene activity and a possible "phase" of nucleosomes (see refs. 1 and 2 for reviews). The method consists in measuring the distance separating DNA ends of chromatin fragments from a fixed restriction site. These ends are usually obtained through micrococcal nuclease digestion. If digestion is brief, the existence of multiple potential cutting sites in the internucleosomal region results in ends of variable lengths, which reflect the sequence specificity of cleavage of the nuclease. A more extensive digestion, on the other hand, leads to a release of H1 and to a possible sliding of nucleosomes on the DNA (3,4). A way to overcome these problems is to subsequently trim the ends of chromatin fragments with exonuclease III (exo III) from E. coli, as reported by other authors (5). In this paper, we describe conditions

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in which an accurate trimming with this exonuclease can be achieved.

Exonuclease III trimming of chromatin was first described by Prunell and Kornberg (6,7) and Riley and Weintraub (8) and has previously been applied to nucleosome dimers to analyse the center-to-center distance of nucleosomes in rat liver chromatin (7,9). These investigations led to the qualitative conclusion that the nucleosome repeat length varies inside a single cell as well as among different organisms or different tissues of the same organism (see ref. 10 for a review). In preliminary studies (7), however, the contribution of a possible sliding of nucleosomes to the heterogeneity of DNA in trimmed dimers could not be determined. In subsequent investigations (9), the sliding problem was obviated by using H1-containing dimers instead of H1-depleted ones. A uniform trimming could, however, not be achieved, so that overtrimmed dimers, which had lost H1, had to be excluded from the measurements.

We describe here an improved method which led to a uniform trimming of DNA in nucleosome oligomers. This trimming was performed down to the first major impediment encountered by the exonuclease, which arises from the interaction of H1 at the entrance and exit of the DNA in the nucleosome. Such a trimming was applied to nucleosome dimers and trimers and allowed us to investigate the arrangement of nucleosomes in bulk rat liver chromatin.

MATERIALS AND METHODS

Materials. Rats were male Wistar. Micrococcal and S1 nucleases were purchased from Sigma. Exonuclease III and T4 polynucleotide kinase were from New England Biolabs. Bacterial alkaline phosphatase was from Worthington and γ^{32}P-ATP from Amersham.

Nuclei and chromatin. Rat liver nuclei were prepared according to the procedure of Blobel and Potter (11), except that TKM buffer was replaced by buffer A of Hewish and Burgoyne (12), supplemented with 2.5 mM EDTA. Nuclei were washed in buffer A containing 0.34 M sucrose and 0.1 mM EDTA and finally resuspended (0.1 ml/g of liver) in the same buffer to which was added 10 mM

Na bisulfite, pH 7.5.

Nuclei were digested with micrococcal nuclease (6 U/ml ; 2 min at 37°) as previously described (7). Digestion was stopped by adding EDTA, pH 7.5, to 10 mM. After centrifugation, nuclei were lysed by resuspension in 0.2 mM EDTA, pH 7.5, and 0.2 mM PMSF (Phenyl Methyl Sulfonyl Fluoride) at 0°. Debris was spun down and the chromatin supernatant recovered.

Chromatin precipitation in 0.15 M salt. This was performed essentially as previously described (9). Chromatin, at a DNA concentration of 1 mg/ml, was incubated 2 min at 0° in 0.15 M NaCl. After spinning for 5 min at 10,000 g, chromatin was resolubilized in 1 mM Tris-HCl, 0.1 mM EDTA, pH 7.5, and 0.2 mM PMSF. The recovery was about 80 %. The high chromatin concentration ensures the virtually complete precipitation of monomers and dimers containing one and two Hls, respectively, as shown in the "nucleoprotein" gel of fig. 1, where chromatin before and after precipitation are compared. Fig. 1 also shows that very few 1H1 dimers and no H1-lacking monomers precipitate.

Exonuclease III digestion of chromatin. Chromatin, at a DNA concentration of 250 µg/ml, in 1 mM Tris-HCl, 0.25 mM $MgCl_2$, 0.1 mM EDTA, 1 mM β-mercaptoethanol and 0.2 mM PMSF, pH 7.5, was digested with exo III for 5 min at 37°. Digestion was stopped by the addition of EDTA, pH 7.5, to 0.25 mM.

Gel electrophoresis of chromatin. Chromatin was fractionated by electrophoresis in polyacrylamide gels as described (13) except that the electrode buffer was 1 mM Tris-HCl, 0.1 mM EDTA, pH 7.5. The gel was preelectrophoresed and the buffer extensively recirculated between the two reservoirs.

Gel electrophoresis of DNA. DNA was electrophoresed in polyacrylamide gels either in duplex form (14) or as single strands in the presence of 98 % formamide (15,16) or 7 M urea (16)

Other methods. DNA was eluted from "nucleoprotein" gels as described (17). After elution, DNA was prepared by adjusting the solution to 1 M NaCl and 1 % SDS, extraction with 1 volume of chloroform isoamyl-alcohol (24:1 ; v/v) and ethanol precipitation

Terminal labelling of DNA with T4 polynucleotide kinase and γ^{32}P-ATP was performed after dephosphorylation with bacterial alkaline phosphatase (when needed), as described (18).

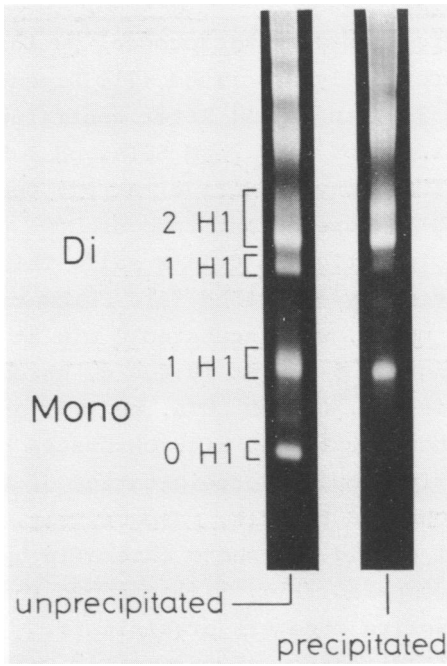

Figure 1. Analysis of chromatin precipitated in 0.15 M salt. Chromatin was prepared from rat liver nuclei and precipitated in 0.15 M NaCl as described in Materials and Methods. After resolubilization, chromatin was electrophoresed in a 4 % polyacrylamide gel together with total chromatin. The gel was stained with ethidium bromide and photographed.

S1 nuclease digestion was performed in 30 mM Na acetate, 0.25 M NaCl, 0.5 mM $ZnCl_2$, pH 4.6, for 30 min at 37°, with 40 U/ml of enzyme. Interestingly, the concentration of S1 nuclease which ensured an optimum trimming of single-stranded ends was found not to depend on DNA concentration, from a few ng/ml to 50 µg/ml.

RESULTS

The method used to trim chromatin with exo III is outlined in fig. 2. 3' ends of DNA in chromatin are first digested with exo III. DNA is then extracted and remaining 5' single-stranded ends are subsequently removed by digestion with S1 nuclease.

Chromatin was prepared by moderate digestion of rat liver

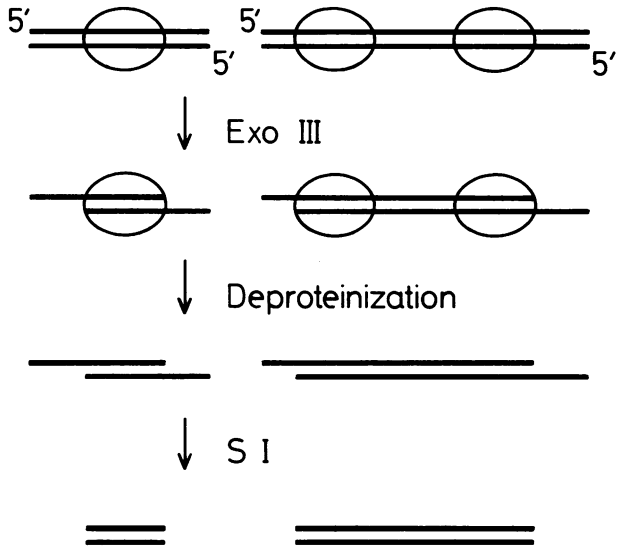

Figure 2. Exonuclease III trimming of chromatin. Diagram of the experiment. DNA 3' ends are first trimmed by incubating chromatin with exo III. DNA is then extracted and 5' single-stranded ends are removed by digestion with S1 nuclease. Ovals represent H1-containing nucleosomes.

nuclei with micrococcal nuclease. Since the interaction of H1 with the nucleosome is to be used to stop the progression of exo III (see Introduction), H1 depleted monomers, together with dimers which have lost one H1, were removed owing to their solubility in 0.15 M salt, in contrast with the insolubility of chromatin with its full complement of H1 (see Materials and Methods and fig.1). After resolubilization of the chromatin, fractions were digested with various amounts of exo III, and separated in two halves. The first half was directly electrophoresed as nucleoprotein in a polyacrylamide gel (fig. 3B). DNA extracted from the second half was digested with S1 nuclease and electrophoresed in another polyacrylamide gel (fig. 3A). The "DNA" gel (fig. 3A) shows that mononucleosomal DNA is progressively shifted from a broad distribution (lane a) to a sharp band at about 160 bp (lane f), in contrast to oligomer bands which remain broad at all stages of digestion. The "chromatin" gel (fig. 3B) shows little change upon digestion, except that the 1H1

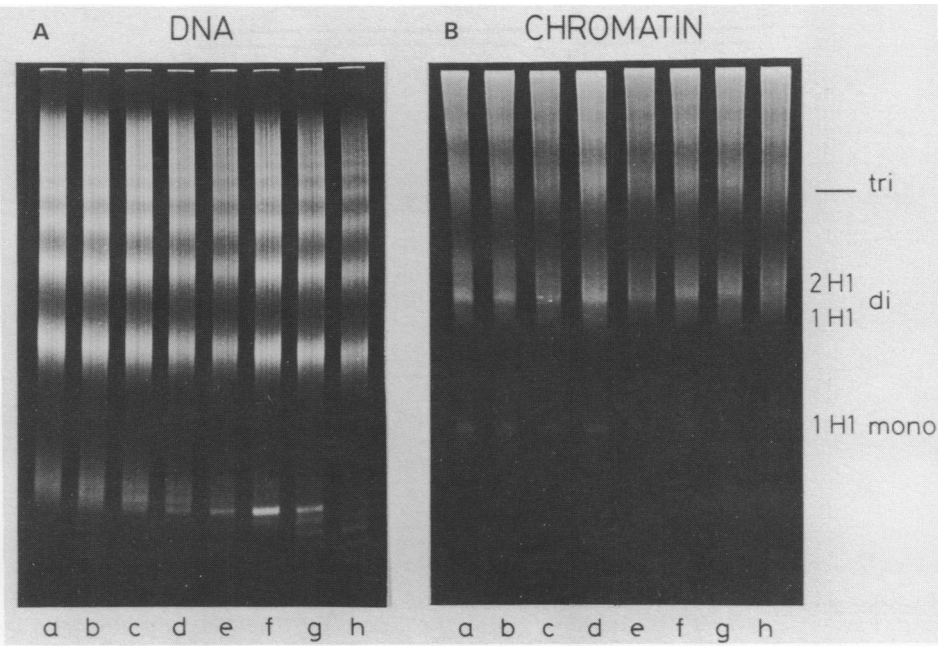

Figure 3. Fractionation of exonuclease III-digested chromatin and DNA. After precipitation in 0.15 M NaCl and resolubilization (see fig. 1) chromatin fractions were digested with the following concentrations of exo III : a:0, b:10, c:18, d:32, e:57, f:100, g:177 and h:320 U/ml, and separated into two halves.

A. DNAs extracted from the fractions were digested with S1 nuclease and electrophoresed in a 4 % polyacrylamide gel.

B. Chromatin fractions were electrophoresed, as nucleoproteins, in a 4 % polyacrylamide gel, as described in Materials and Methods.

Both gels were stained with ethidium bromide and photographed.

monomer band disappears beyond lane f, as also observed for the 160 bp band. Such features are in agreement with the property of exo III-generated 160 bp particles to release H1 upon further digestion (9).

Monomers in lane f of fig. 3 are reduced to homogenous 160 bp particles, i.e. chromatosomes ; dimers and trimers may similarly correspond to two and three chromatosomes, respectively, connected by one and two linkers. DNA length distribution in dimers (see lane f in fig. 3A) would then show the length heterogeneity of the linkers whereas that in trimers would reflect

their arrangement along the chromatin fiber. However, for this to be true, three conditions have to be met. First, no rearrangement of nucleosomes must occur during chromatin preparation and/or digestion. Second, oligomers must be, like monomers, uniformly trimmed at both ends. Finally, 160 bp particles generated by exo III must be "symmetrical", like those (the chromatosomes) produced by micrococcal nuclease digestion, which have, when compared to core particles, 10 bp more DNA at each end (19). If 160 bp particles are asymmetrical and have, for example, 20 bp extra DNA at one end, the other one being at the core position, the length of the DNA in trimmed oligomers would depend on the particular combination of ends, 0 and 0, 0 and 20 or 20 and 20 bp, respectively.

Comparison between the exo III-generated 160 bp particle and the chromatosome. The resemblance of exo III-generated 160 bp particles with chromatosomes suggested that both exo III and micrococcal nuclease stop at the same position. That this was the case was demonstrated by an analysis of the DNA in nucleosome monomers which were fractionated in the "nucleoprotein" gel described above (fig. 3B). The 1H1 monomer band was cut out from two lanes of the gel. One lane (a) corresponded to control chromatin, the other one (f) to monomers optimally trimmed with exo III. DNAs were purified and an aliquot of the exo III-trimmed DNA was digested with S1 nuclease. All DNAs were then terminally labelled with ^{32}P using polynucleotide kinase, mixed with labelled restriction fragments, and electrophoresed in a 98 % formamide-8 % polyacrylamide gel, together with size markers. An autoradiogram of the gel is shown in fig. 4. The control (lane a ; no exo III and no S1) shows a broad band with a mean of 190 bp, whereas the exo III-digested fraction (lane b) exhibits a band at about 160 bp and a higher trailing. Subsequent S1 nuclease digestion (lane c) results in a sharp 160 bp band, as already shown (lane f of the "DNA" gel in fig. 3A).

The presence of a band at about 160 bp in monomers whose DNA 3' ends were trimmed with exo III (see fig. 4), shows that some of the opposite 5' ends of the same strands were already digested to a pause by micrococcal nuclease. This pause can only be located at the entrance of the chromatosome, that is at the

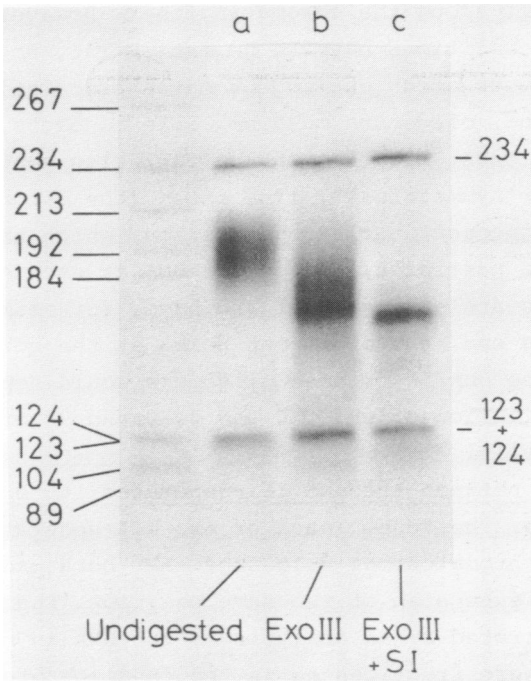

Figure 4. Length distribution of DNA in nucleosome monomers. 1H1 monomer bands were cut out from lanes a and f in the "nucleoprotein" gel shown in fig. 3B. Half of the DNA purified from lane f was digested with S1 nuclease. This DNA (Exo III + S1), together with the remaining half (Exo III), and DNA from lane a (Undigested), were labelled with ^{32}P at their 5' ends, mixed with 123+124 and 234 bp DNA fragments (from a Hae III digest of pBR322 DNA ; see ref. 22), and electrophoresed in a 98 % formamide-8 % polyacrylamide gel, along with total Hae III-digested pBR322 DNA. An autoradiogram is shown.

"160 position". This is further explained in the scheme of fig. 5, where monomers were classified according to their degree of trimming with micrococcal nuclease. Monomer 1 has long ends, monomer 2 is trimmed to the 160 position at one end and monomer 3 at both ends. The amount of monomer 3 is negligible in the material analysed in fig. 4, as indicated by the absence of the 160 bp band in the control. [This must arise from the loss of H1 from chromatosomes during digestion in nuclei.] Upon exo III digestion, monomer 1 contributes only to the trailing. In contrast, monomer 2 contributes half to the trailing and half to the

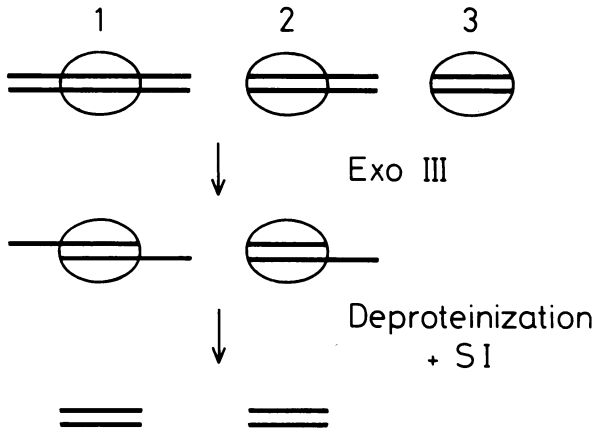

Figure 5. Exonuclease III and S1 nuclease digestions of different species of nucleosome monomers. Nucleosome monomer 1 has long DNA ends. Monomers 2 and 3 have one end and both ends, respectively, digested to the 160 bp position by micrococcal nuclease. The amount of monomer 3 is negligible (see Results). Upon exo III trimming to the 160 bp position, monomer 1 produces DNA of variable length, whereas monomer 2 contributes to half of the 160 bp band. S1 nuclease digestion results only in 160 bp fragments. Ovals represent H1-containing nucleosomes.

160 bp band. From the relative intensities of the 160 bp band (about 25 % of the total ; fig. 4, lane b), monomers 1 and 2 can be estimated to be present in about equal amounts. The band seen in exo III-digested monomers (fig. 4, lane b) contains fragments with one exo III end and one micrococcal nuclease end. That the size of these fragments is close to 160 nucleotides indicates that both exo III and micrococcal nuclease pauses are approximately coincident. The 160 bp particle generated by exo III therefore resembles the chromatosome (19), and is symmetrical.

Control of the trimming of nucleosome oligomers. In contrast to monomers, trimming of oligomers cannot be directly assessed since linker heterogeneity contributes to the width of the bands. Two lines of evidence suggest, however, that oligomers, and more specifically dimers, are uniformly trimmed to the 160 bp position at the same time as monomers. These evidences lie, first, on the study of the dependance of the width and of the mean of the dimer band on the extent of exo III digestion, and, second, on the

observation that a subset of the oligomers contain nicks in the linker whose 3' termini are digested with exo III.

The width of the length distribution of dimer DNA was measured using DNAs extracted from the 2H1 dimer bands of the "nucleoprotein" gel described above (fig. 3B), which corresponded to chromatin fractions digested with various amounts of exo III. Aliquots of the purified DNAs were digested with S1 nuclease, terminally labelled with ^{32}P, mixed with labelled restriction fragments, and electrophoresed in a 98 % formamide-6 % polyacrylamide gel, along with size markers. An autoradiogram of the gel (fig. 6) shows, in addition to the upper dimer band, some lower material in the monomer region. [The origin of this material will be investigated below.] Means and standard deviations of dimer DNA length distribution were measured on the Gaussian-like peaks obtained in traces of the autoradiogram of fig. 6 and were plotted in fig. 7 as a function of exo III digestion. [A trace corresponding to the best trimmed dimers is shown in fig. 10.]

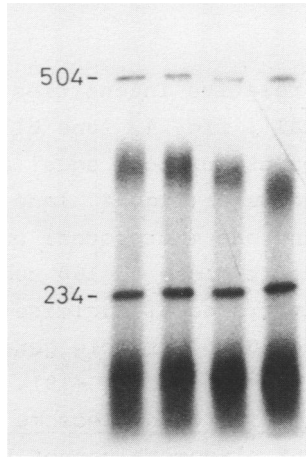

Figure 6. Length distribution of DNA in nucleosome dimers. 2H1 dimer bands were cut out of the "nucleoprotein" gel shown in fig. 3B. After purification, DNAs were digested with S1 nuclease, labelled at their 5' termini with ^{32}P, mixed with two restriction fragments from a Hae III digest of pBR322 DNA (22), and electrophoresed in a 98 % formamide-6 % polyacrylamide gel. An autoradiogram is shown. DNA fractions (from left to right) originate from lanes a, d, f and h of the gel of fig. 3B, respectively.

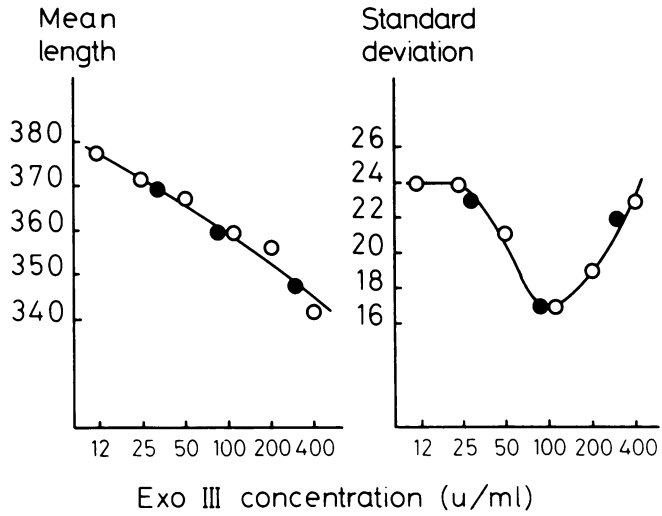

Figure 7. Mean and standard deviation of the length distribution of dimer DNA as a function of exonuclease III digestion. Mean and standard deviation (in nucleotides) were determined from traces of the autoradiogram of fig. 6 (●) and of other autoradiograms corresponding to similar experiments not shown here (o).

The standard deviation (fig. 7) is maximum for untrimmed dimers, decreases upon digestion to reach a minimum value at the level of the best trimming of monomers (100 exo III U/ml ; see fig. 3A), and then increases upon further digestion. In contrast to the width, the mean length (fig. 7) decreases almost linearly upon digestion. However, its value at the level of the best trimming of monomers, that is, at the minimum value of the width, leads to the expected average length of the linker (see below). These features indeed suggest that dimers are, like monomers, uniformly trimmed to the 160 bp position.

The second evidence in favor of a good trimming of dimers comes from the analysis of the distribution of monomer-size fragments seen in the gel of fig. 6. These fragments do not result from aggregates of two monomers comigrating with dimers in the nucleoprotein gel of fig. 3B. This was shown as follows : Dimer DNAs purified from the "nucleoprotein" gel of fig. 3B (lane f) were terminally labelled with ^{32}P (without prior melting) and electrophoresed in both a 4 % polyacrylamide and a 98 %

formamide-6 % polyacrylamide gel in their native and denatured form, respectively. Autoradiograms (not presented) showed the presence of monomer-size material only in the second gel, therefore demonstrating that this material arises from nicks present in the linker of some of the dimers. These nicks were not introduced by exo III since the amount of this material does not depend on the extent of digestion with the enzyme (see fig. 6). Length distribution of monomer-size fragments was further investigated by electrophoresis of the same DNAs in a 7 M urea-8 % polyacrylamide gel. An autoradiogram of the gel (fig. 8) shows that these fragments are resolved into bands which resemble those seen in the monomer region of the "DNA" gel of fig. 3A ; in particular, the same sharp 160 nucleotide band is seen (fig. 8, third lane from the left), although on a higher background. Interestingly, a similar analysis of trimer DNA extracted from the "nucleoprotein" gel of fig. 3B also revealed a sharp 160 nucleotide band at the same level of digestion (not shown). The origin of this band is explained in the scheme of fig. 9, which shows a dimer with one nick in its linker. Upon incubation with exo III, all 3' DNA termini are digested to the 160 bp position. Subsequent S1 nuclease digestion separates the two nucleosomes

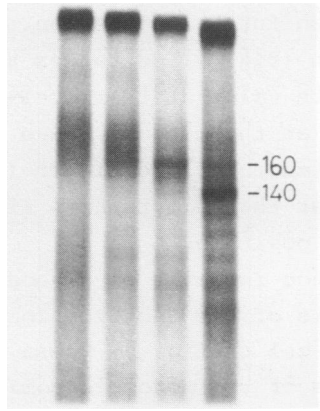

Figure 8. Length distribution of DNA in nucleosome dimers. DNAs, identical to those of fig. 6 (except that they were not mixed with marker fragments), were electrophoresed in a 7 M urea-8 % polyacrylamide gel. An autoradiogram is shown.

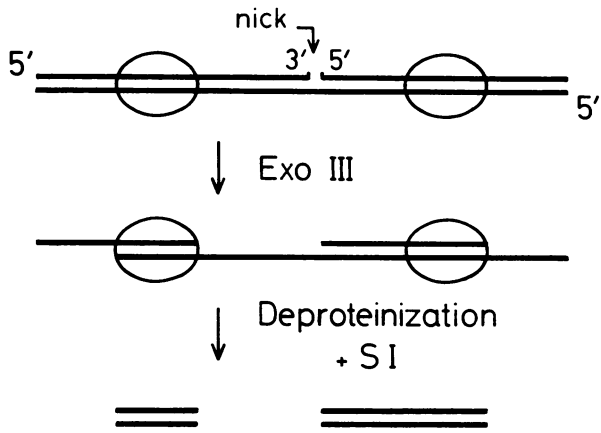

Figure 9. Exonuclease III and S1 nuclease digestions of a nicked nucleosome dimer. Upon exo III digestion, all DNA 3' ends are digested to the 160 bp position. Upon further S1 nuclease digestion, nucleosome 1 (left) results in 166 bp fragments, whereas nucleosome 2 (right) gives DNA fragments of variable lengths. Ovals represent H1-containing nucleosomes.

from each other ; the first one (left in fig. 9) contributes to the 160 bp band and the second one to the background. This therefore indicates that nicked dimers and trimers are, like monomers, uniformly trimmed to the 160 position.

That both the width and the mean of dimer DNA length distribution vary beyond the point at which dimers are uniformly trimmed to the 160 bp position (fig. 7) may appear surprising. This results from the failure of dimers to release H1 when overtrimmed, in contrast to monomers, as revealed by both the lack of enrichment of the 1H1 dimer band upon exo III digestion (see fig. 3B) and the appearance of a 140 bp band in the DNA distribution of dimers (right in fig. 8). Such an unexpected stability of H1 might be due to its residual interaction with the central linker.

Length distribution of the linker. Mean lengths of monomer (from fig. 4) and dimer (from fig. 6) DNAs are listed in table 1. Only values corresponding to the control and to the best trimmed fraction are given. Since trimmed dimers and monomers have the same ends (which is not necessarily the case before trimming) their length difference should be equal to the chromatin repeat

Table 1. Mean and standard deviation of length distribution of DNA from nucleosome monomers and dimers.

	Undigested	Exo III + S1
2H1 dimers	380 (22)	360 (17)
1H1 monomers	190 (12)	162 (3)
	nucleotides	

Data refer to 2H1 dimers and 1H1 monomers which were excised from lanes a (Undigested) and f (Exo III + S1) of the "nucleoprotein" gel of fig. 3B. Lengths were measured, using size-markers, from the Gaussian-like peaks recorded from autoradiograms of fig. 4 (1H1 Mono) and fig. 6 (2H1 Di) (Undigested and Exo III + S1 ; first and third lanes from the left, respectively). The precision of the measurements was estimated to be ±1 %.

length. A value of 198 bp (360-162 ; see table 1) is found, in good agreement with published values of 196 bp (20) and 198 bp (21) for the repeat of rat liver chromatin. It is noteworthy that this agreement argues against any sliding of nucleosomes, either during preparation or digestion of chromatin.

As shown from traces of monomer and dimer bands, all DNA length distributions are approximately Gaussian (see, in fig. 10, the length distribution of DNA in trimmed dimers). This justifies the introduction of the standard deviation of the distributions, which is also listed in table 1. The mean length (m) of the linker, which is defined here as the intercore DNA, is 52 (198-146) bp. The standard deviation of its distribution (σ) is the same as that of trimmed dimers, i.e. 17 bp (see table 1). We can therefore estimate that 70 % of the linkers have a length comprised between 35 bp ($m-\sigma$) and 69 bp ($m+\sigma$), and 95 % between 18 bp ($m-2\sigma$) and 86 bp ($m+2\sigma$). In other words, DNA content per nucleosome, of average 198 bp, ranges between 181 and 215 bp for 70 % of them, and between 164 and 232 bp for virtually all of them.

Linker arrangement along the chromatin fiber. As mentioned above, whereas DNA distribution in trimmed dimers only revealed the length heterogeneity of the linkers, trimmed trimer DNA distribution reflects the arrangement of these linkers along the chromatin fiber. If linkers were arranged at random, then the

width of the length distribution of the DNA would increase with the square root of the number of linkers in each oligomer. The trimer DNA band would then be $\sqrt{2}$ times broader than the dimer DNA band. We may also envisage non random arrangements such as a clustering of short or long linkers with each other which would broaden the trimer band ; in contrast, alternating short and long linkers would narrow it.

We investigated the linker arrangement as follows. Nucleosome trimers were cut out from lane f of the "nucleoprotein" gel of fig 3B, the DNA extracted, digested with S1 nuclease, and terminally labelled with ^{32}P as above. The labelled DNA was then electrophoresed in a 98 % formamide-6 % polyacrylamide gel along with size markers, and the gel was autoradiographed (not shown). A trace of the trimer band is displayed in fig. 10, together with a trace of the dimer band (taken from fig. 6) corresponding to the same optimal level of exo III digestion. Such a comparison shows that length distribution of trimer DNA is similar to that

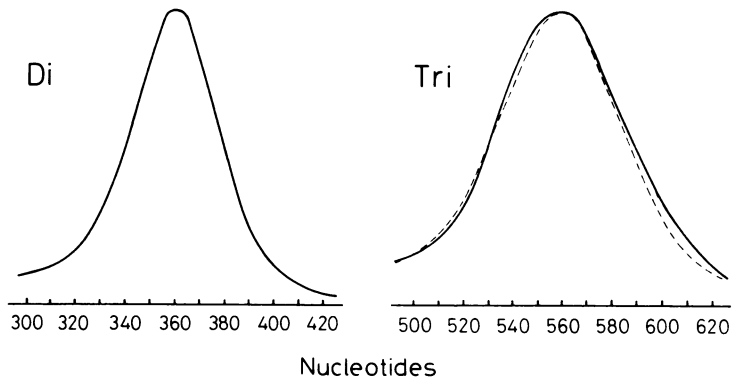

Figure 10. Length distribution of DNA in trimmed dimers and trimers. The trace of trimmed dimers was recorded from the autoradiogram of fig. 6 (third lane from the left). The trace of trimmed trimers was obtained from DNA which was purified from lane f of the gel of fig. 3B and subsequently digested with S1 nuclease, labelled with ^{32}P and electrophoresed, as described in Results. Trimer DNA was calibrated against fragments from a Hpa II digest of pBR322 (22). Length distribution of trimer DNA which would be obtained if linkers were randomly arranged along the DNA is indicated by the broken line. This statistical distribution was derived from the dimer peak by shifting it by 200 bp (the difference in the means of trimers and dimers) and by multiplying its width by $\sqrt{2}$.

calculated from dimers assuming a statistical arrangement of the linkers (broken line in fig. 10).

Given the failure to fractionate trimers with 0, 1, 2 or 3 H1s, the question may be asked whether most of trimers in the band which was cut out of the gel had actually kept their full H1 complement. Although this question cannot be directly answered, this is in fact likely in view of the mean length of trimmed trimers, which is 200 bp higher than that of trimmed dimers (see fig. 10). The absence of trailing on the lower side of the trimer peak further supports this view.

DISCUSSION

Trimming of the DNA at the ends of chromatin fragments, which is described here, makes use of the first large impediment encountered by exo III in its digestion of chromatin. This impediment is identical, within one or two nucleotides, to that encountered by micrococcal nuclease (see Results) at the 160 bp position, which results from the interaction of H1 at the entrance and exit of DNA in the nucleosome (21,23). The second large impediment, which is located at the core position and which does not involve H1, may not be used for these experiments, because nucleosomes which have lost H1 may no longer be locked into position and may slide toward each other.

Only trimming of monomers can be quantitated in a straightforward way. Trimming of dimers and trimers could, however, be assessed and was shown to be synchronous with that of monomers. This demonstration made use of the preexisting nicks in the linkers of some of the oligomers, whose 3' ends are also digested with exo III to the internal 160 bp position. Subsequent S1 nuclease digestion destroys the single-stranded DNA facing the gap and reveals a sharp 160 bp band. Additional support for a synchronous trimming of monomers and dimers comes from the observation that the width of the dimer band reaches a minimum and the mean, its expected value (see fig. 7), when monomers are optimally trimmed. The question remains open, however, whether exo III also trims large oligomers at the same rate, since the possible induction of a superstructure by Mg^{2+} may interfere with the trimming process.

The lowest amount of DNA per nucleosome (164 bp ; see Results) is actually close to that found in a chromatosome (166 bp), which is the minimum required to keep H1 bound (21). Such a result was not unexpected since the procedure was, and in fact could only be, applied to dimers which contain their full complement of H1. For the same reason, this result is not relevant to the question whether all nucleosomes in chromatin contain H1. In contrast, the maximal DNA content (232 bp ; see Results) is larger than any repeat length known so far in somatic cells. It is smaller, however, than repeats found in the sperm of sea urchins (240, 250 and 260 bp ; see refs. 24, 25 and 26). The exceptionally high repeats of sperm might, however, be due to some specific features (such as particular types of H1 ; see refs. 27 and 28), which do not apply to somatic cells. If this is true, 90 bp of linker DNA might then be close to the limit length which can be accomodated between any two nucleosomes of the fiber without disruption of its higher order structure.

The present result that linker arrangement is statistical is in contrast to the previous conclusion of Garrard and co-workers (29), based on electron microscopic examination of chicken erythrocyte nucleosome trimers, that adjacent linkers share common DNA lengths. It is not clear whether this discrepancy is only due to the different origin of the chromatin.

The present result formally demonstrates that linker lengths vary from one nucleosome to the next along a single chromatin fiber, and not solely from one cell to another. This conclusion had only been reached before from the study of the chromatin structure of particular DNA sequences.

It is noteworthy that a statistical arrangement of linkers is consistent with the idea of an overall random location of nucleosomes along the bulk of the DNA. This was first suggested by the failure to observe, using DNA reassociation, a unique relation between the position of nucleosomes and the nucleotide sequences of single copy DNA (6,7,30).

We must finally emphasize that our conclusion that linkers of different lengths are statistically interspersed in rat liver refers to bulk chromatin. It is clear that linkers of a given length might be clustered in some particular sequences, as

several reports have already shown (see refs. 1 and 2 for reviews) This work only demonstrates that such situations are not frequent enough to be detected by our analysis.

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