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Alignment of nucleosomes along DNA and organization of spacer DNA in *Drosophila* chromatin

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ABSTRACT

A series of mono- and dinucleosomal DNAs characterized by an about ten-base periodicity in the size were revealed in the micrococcal nuclease digests of Drosophila chromatin which have 180±5 base pair (bp) nucleosomal repeat. 20, 30, and 40 bp spacers were found to be predominant in chromatin by trimming DNA in dinucleosomes to the core position. Among several identified mononucleosomes (MN), MN_{170}, MN_{180} and MN_{190} were isolated from different sources (the figures indicate the DNA length in bp). The presence of the 10, 20, and 30 bp long spacers was shown in these mononucleosomes by crosslinking experiments. The interaction of histone H3 with the spacer in the Drosophila MN_{180} particle was also shown by the crosslinking /5/. We conclude from these results that the 10 n bp long intercore DNA (n=2, 3 and 4) is organized by histone H3, in particular, and together with the core DNA forms a continuous superhelix. Taken together, these data suggest that Drosophila chromatin consists of the regularly aligned and tightly packed MN_{180}, as a repeating unit, containing 10 and 20 bp spacers at the two ends of 180 bp DNA. Within the asymmetric and randomly oriented in chromatin MN_{180}, the cores occupy two alternative positions spaced by 10 bp.

INTRODUCTION

An increased interest in the chromatin structure arises from the belief that it is directly related to the genome functioning. Nucleosomes representing the first level of the chromatin organization are aligned along DNA in a 10-nm fibril which is further packed into an about 25-nm chromatin fiber /1, 2/. The core particle is a basic element of nucleosomes. The particle if a flat disc (11x11x5.7 nm) in which 146 base pairs (bp) of DNA are wound in about 1.75 turns of a left--handed superhelix around a histone octamer /3/. The histone octamer is composed of two molecules of each of the histones

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H2A, H2B, H3, and H4. Nucleosomes incorporate the core particle and contain, in addition, histone H1 and the intercore DNA, a spacer, of variable length. The sequential arrangement of histones along DNA was determined in the core particle and in some nucleosomes /4, 5/. The specific location of nucleosomes within a number of DNA sequences in chromatin, i.e. nucleosome phasing, has been demonstrated by taking an advantage of the essentially enhanced suscessibility of the spacer in comparison with core DNA to the action of micrococcal nuclease /6-12/.

This paper demonstrates, as a result of studies of mononucleosomes, dinucleosomes and their DNA, the approximately 10 bp periodicity in the length of the intercore DNA and suggests the role of histone H3 in its organization probably as superhelical extension of the core DNA. We also propose here the model of chromatin structure accomodating the heterogeneous spacer length into regularly aligned nucleosomes which contain the DNA of the same nucleosomal repeat size.

METHODS

Analysis of the nuclease digests of chromatin. Nuclei and chromatin were isolated from dechorinated *Drosophila melanogaster* embryos (6-18 h) and rat liver as described /4/. Chromatin was prepared from sea urchin (*Strongylocentrotus intermedius*) sperm according to /13/. The nuclei and chromatin (20 A_{260} units/ml) were digested with micrococcal nuclease (Worthington) at a concentration of 30 or 12 u/ml, respectively, in 10 mM Tris-Cl, pH 8.0, 0.3 mM $CaCl_2$, 0.5 mM phenylmethylsulfonyl fluoride at 37°C. The digestion of the nuclei (20 A_{260} u/ml) with DNAase I (10 u/ml, Worthington) was carried out in 10 mM Tris-Cl, pH 8.0, 10 mM NaCl, 3 mM $MgCl_2$ at 37°C for 10 min. The digestion was terminated by adding 4 mM EDTA and 0.5% SDS. DNA was proteinized by treatment with pronase and cetyltrimethyl ammonium precipitation /4/, and then was electrophoresed in non-denaturing 1.5% agarose slab gel (150x x150x3 mm) in 90 mM Tris-borate, pH 8.3, 2.5 mM EDTA at 100 V or in denaturing 6% acrylamide slab gel (180x180x1 mm) in the same buffer containing 7 M urea at 350 V /4/. The gels were

stained with ethidium bromide.

The length of the mono- and oligonucleosomal DNA was measured by gel electrophoresis from semi-logarithmic plot of the size of the markers (DNAase I produced fragments of nuclei or HaeIII restriction fragments of RF ØX174 DNA) versus the distance of their migration in the gels.

Trimming DNA in dinucleosomes to the core position. This was carried out according to Prunell and Kornberg /14/ with the whole micrococcal nuclease digest of Drosophila nuclei without an intermediate isolation of dinucleosomes as described in /10/. The digest (3 A_{260} units/ml) was hydrolyzed with exonuclease III (10 units/ml) in 1.2 mM $MgCl_2$, 1 mM mercaptoethanol, 66 mM Tris-Cl, pH 8.0, at 37°C for 45 min. DNA after isolation was digested with endonuclease S_1 (60 units/mg DNA) in 0.1 mM $ZnCl_2$, 25 mM Na acetate, pH 5.0, for 30 min at 20°C, and then electrophoresed.

Location of histone binding sites on DNA in nucleosomes. This was carried out as described /4/ with a modification: free single-stranded DNA was removed from the crosslinked histone-DNA complex by their binding to a hydroxylapatite column /15/ and the elution of free DNA from the column with 0.15 M Na-phosphate, pH 6.4, 0.1% SDS.

RESULTS

A series of mono- and dinucleosomal DNAs with a ten-base periodicity in the length. A number of histone H1-containing nucleosomes /16/ differing in the size of the spacer by about 10 bp /5/ has been effectively separated by gel electrophoresis of the micrococcal nuclease digest of chromatin. The use of gel electrophoresis of the denatured DNA from the digest instead of the electrophoresis of either nucleosomes or native DNA has provided a higher resolution of the DNA heterogeneity. It has revealed the mononucleosomes (MN) MN_{170}, MN_{180}, and MN_{190} described previously as MN_{165}, MN_{175}, and MN_{185}, respectively /5/. The subcripts show the DNA length in bp. Here we extend these studies for other mononucleosomes and dinucleosomes.

Fig. 1 shows the gel electrophoresis of denatured DNA fragments isolated from the micrococcal nuclease digest of chromatin and nuclei. A regular ladder of discrete DNA bands in the gels extends beyond the level of mononucleosomal DNA and includes the DNA of nucleosomal dimers (Fig. 1A, e,g) as well as the DNA in the region between them (Fig. 1A, a). This indicates that the core and spacer DNA are regularly sized. The regularity of the ladder corresponds to that of DNAase I generated fragments which can be discerned up to the level of the dinucleosomal DNA (Fig. 1A, b; 1B, d). Moreover, in the earliest micrococcal digests of nuclei the mobility of the mono- (Fig. 1B, a) and even dinucleosomal DNA fragments (Fig. 1B, c) coincides well with that of the fragments produced by digestion of rat liver (Fig. 1B, b) as well as Drosophila embryo

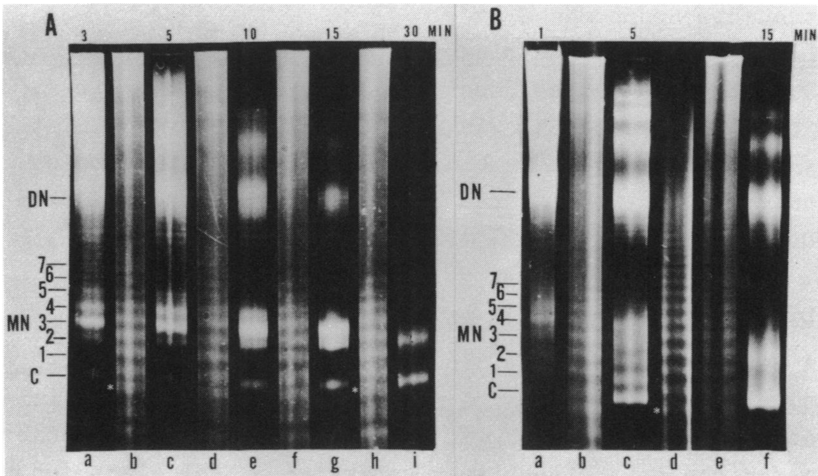

Fig. 1. A regular series of DNA fragments in the micrococcal nuclease digests of chromatin (A) or nuclei (B). DNA was isolated from the digests (time of digestion is shown in min), denatured, then electrophoresed in 6% polyacrylamide gel for 2 h in the presence of 7 M urea and stained with ethidium bromide. The single-stranded DNA fragments produced by digestion of rat liver nuclei (Fig. 1A,b,d,f,h; Fig. 1B,b,e) or Drosophila nuclei (Fig. 1B, d) with DNAase I were electrophoresed in parallel lanes as markers; the asterics denotes the DNA fragment of 142 bases in length, the lengths of the following fragments upward are 154, 166, 176, 184, 194, 202, and 212 bases /18/. MN and DN are mononucleosomal and dinucleosomal DNA.

nuclei (Fig. 1B, d) with DNAase I. The length of the DNAase I generated fragments from rat liver nuclei is a multiple of about 10 bases /17/; the fragments were precisely sized /18/ and used here as markers to measure the nucleosomal DNA lengths that are summarized in Table 1. This coincidence in the size of the micrococcal nuclease and DNAase I generated fragments over the region of mono- and dinucleosomal DNA indicates that the length of the core as well as the spacer DNA

Table 1. The length of mononucleosomal and dinucleosomal DNA in the micrococcal nuclease digests of Drosophila chromatin and nuclei.

Mononucleosomes	Chromatin[a] (Fig.1A)					Nuclei[b] (Fig.1B)		
	Digestion time (min)							
	1	3	5	15	30	1	5	15
Core	153	151	150	148	146	154	148	146
MN1 (MN_{160})	166	162				166	160	160
MN2 (MN_{170})	176	174	173	170	168	176	172	169
MN3 (MN_{180})	184	182	179	180	179	184	180	
MN4 (MN_{190})	196	193	192	189		194	190	
MN5 (MN_{200})	208	204	200			202	200	
MN6 (MN_{210})	214	210	210			212		
MN7 (MN_{220})	220					220		

Dinucleosomes	Nuclei[b] (Fig.2)		
	Digestion time (min)		
	1	15	1 (Exo III + S_1)
DN1 (DN_{320})	320	318	318
DN2 (DN_{330})	332	332	330
DN3 (DN_{340})	342	342	340
DN4	360	355	
DN5	375	370	
DN6	385	380	

The lengths are an average of 5 (a) or 2 (b) experiments. The scatter in the values for mono- and dinucleosomal DNA is about ±2 and ±4 bases, respectively. (Exo III + S_1) - the nucleosomal DNA in the nuclei digest for 1 min was trimmed with exonuclease III and endonuclease S_1 (10,14).

should be multiple of the same about 10 bases. It is of interest to note that an additional regularity of about $10n^{+}5$ has been found in the DNAase I digest of yeast chromatin /18/.

At the initial stages of the digestion of Drosophila chromatin (Fig. 1A, a) or nuclei (Fig. 1B, a) with micrococcal nuclease, up to seven bands of mononucleosomal DNA, MN1-MN7, besides the core DNA, are well resolved in the gels. The band of MN1 is rather faint in the chromatin (Fig. 1A) but is quite prominent in the nuclei digest (Fig. 1B, a, c). In the course of digestion the bands of longer DNA, MN3-MN7, disappear one after another due to their apparent conversion into lower nucleosomes, and the mononucleosomal DNAs are shortened by about 5-8 bases (Table 1). This DNA trimming likely results from the exonuclease activity which is present in micrococcal nuclease /19/. Similar sizes of the nucleosomal double-stranded DNA, namely, 146, 157, 168, 178, and 200 bp, were found in the micrococcal nuclease digest of chicken erythrocyte chromatin /20/. In the digest of chromatin from rat liver or sea urchin sperm, the similar series of the DNA bands with the same periodicity extending even up to MN9 and higher was also observed (not shown).

Prolonged electrophoresis of the DNA from the nuclei digest provided the better resolution of six bands of the dinucleosomal DNA (Fig. 2b, d). The size of the fragments was measured by using the HaeIII restriction fragments of RF ØX174 DNA as markers (Table 1). This also demonstrates an approximately ten-bases periodicity for the most of the bands. Some sharp bands seen in Figs. 2b and 2d above the dinucleosomal DNA corresponded to RNA since they disappeared upon treatment with pancreatic RNAase (not shown).

Nucleosomal DNA multiplicity in the digest of the bulk chromatin is unlikely to be due, in general, to structural heterogeneity in different chromatin regions. It follows from experiments in which very similar mononucleosomal DNA gel patterns were revealed for the bulk chromatin (Fig. 1) and for its different regions containing rather short (from 359 to 850 bp) repeated DNA sequences from the silent type I and type II insertions within 28S ribosomal DNA gene and 1.688 satellite

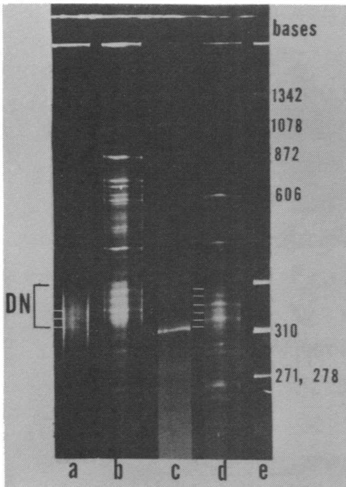

Fig. 2. A regular series of dinucleosomal DNA. DNA from the nuclei digests for 1 min (b) and 15 min (d) was electrophoresed for 4 h as described in Fig. 1. During this prolonged electrophoresis, the mononucleosomal DNA runs off the gel. The 1 min digest (b) was treated with exonuclease III and nuclease S_1 and then DNA was isolated and electrophoresed (a). The single-stranded Hae III restriction fragments of RF ØX174 DNA were electrophoresed as markers (e and e). Three (a) and six (d) thin lines indicate the position of DN1-DN3 and DN1-DN6 respectively.

DNA. In these experiments, DNA after electrophoresis was transferred to DBM-paper /21/ and hybridized with ^{32}P-labeled DNA of plasmids B8, R15 /22/ and pDm23 /23/, respectively (results are not shown).

The conclusion is supported by the finding of the same, 180±5 bp nucleosomal repeat for the bulk chromatin and for these chromatin regions (not shown). This value corresponds to the 180±4 bp long chromatin repeat measured for Drosophila hsp70 gene /24/.

The length of intercore DNA in dinucleosomes is about 20, 30, and 40 bp. Prunell and Kornberg /14/ have shown the size heterogeneity of the spacer DNA in rat liver dinucleosomes by trimming their DNA from the both ends to the position of the cores by sequential digestion with exonuclease III and nuclease S_1. We repeated these experiments with Drosophila dinucleosomes. The whole micrococcal nuclease digest of nuclei was treated with exonuclease III and nuclease S_1 /14, 10/, and then the DNA was electrophoresed. Three rather diffuse bands of the DNA about 320, 330, and 340 bp long are seen in the gel after the trimming (Fig. 2a). The lengths of DNA in the trimmed dinucleosomes and in the three shortest untrimmed dinucleosomes DN_{320}, DN_{330}, and DN_{340} coincide well with each other (Table 1). Taking into account that the dimeric DNA is trimmed

to the core positions and the core DNA is about 150 bp long, one may conclude that the length of the intercore DNA is about 20 (320- 2x150), 30 (330- 2x150), and 40 bp (340- 2x x150). The size of the core DNA is taken here to be 150 bp because this should be length of DNA in the full core particles at the initial stages of the micrococcal nuclease digestion (154 bp, see Table 1) which are trimmed only from one side within dinucleosomes to the level of the 146 bp core particles. The intensities of the three trimmed dimeric DNA bands in the gel are rather similar. This means that the core particles are spaced in chromatin by 20, 30, and 40 bp which are equally frequent.

Comparative location of the histone octamer and histone H3 on DNA in the core, MN_{180} and MN_{190} particles. In the previous reports from this laboratory, the primary organization (the linear order of histones along DNA) of the core, MN_{170} and MN_{180} particles has been described /4, 5/. These particles were isolated from the micrococcal nuclease digests of *Drosophila* embryos and rat liver chromatin. Among different sources, the MN_{190} particles have been isolated in more pure state from sea urchin sperm chromatin. We use this chromatin due to much higher resolution of its H2A and H2B histones by SDS-gel electrophoresis as compared with that of rat liver or *Drosophila* embryo histones. The resolution is important for unambiguous localization of histone H2A and H2B by two-dimensional gel electrophoresis (see below) on some regions of nucleosomal DNA /4, 5/. Gel electrophoresis of the micrococcal nuclease digest of the sperm chromatin resolves five fractions of mononucleosomes (Fig. 3A). The fractions were eluted from the gel and analyzed. All of them contained a normal amount of core histones, and fractions 2-5 contained variable amount of histone H1 (not shown). Electrophoresis of the denatured DNA (Fig. 3B) showed the presence of rather homogeneous DNAs having 146, 180 and 190 bases in length in fractions 1, 2 and 3, respectively, and the fractions thus corresponded to the core, MN_{180} and MN_{190} particles. Fractions 4 and 5 contained heterogeneous DNA fragments (not shown).

The procedure for sequencing the position of histones

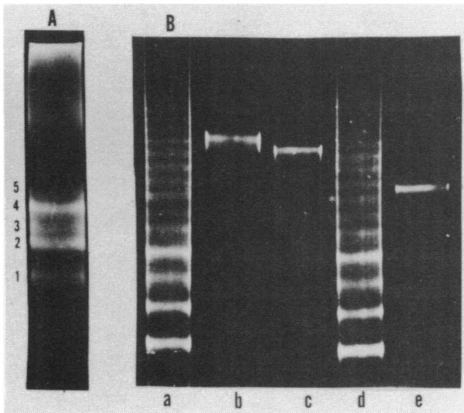

Fig. 3. Electrophoretic separation of sea urchin sperm nucleosomes and analysis of their DNA. (A) - micrococcal nuclease digest of sea urchin sperm chromatin was electrophoresed in 7% polyacrylamide gel (16,5). (B) - mononucleosomes were eluted from the gel and the DNA of the fraction 1 (e), fraction 2 (c) and fraction 3 (b) were electrophoresed under denaturing conditions. DNA fragments from the DNAase I digest of the nuclei were electrophoresed as size markers (a and d). For details see Fig. 1.

along DNA has been described in full detail elsewhere /4/ and therefore is only briefly outlined here. Histones in the sea urchin sperm chromatin were crosslinked to DNA /15/, the chromatin was digested with micrococcal nuclease and nucleosomes were isolated by gel electrophoresis (Fig. 3A). Crosslinks cause single-stranded breaks in DNA in such a way that only the resultant 5'-terminal DNA fragments become attached to protein molecules. Thus, the length of crosslinked 5'-terminal DNA fragments shows the distance of the histone binding site on one DNA strand from its 5'-termini (see Fig. 5 below). The size of ^{32}P-labeled DNA fragments crosslinked in nucleosomes to each histone fraction was determined by two-dimensional gel electrophoresis which separates the DNA fragments attached to different histone fractions into several diagonals and simultaneously fractionates the fragments according to their length.

Fig. 4 shows such two-dimensional gels for the core, MN_{180} and MN_{190} particles isolated from sea urchin sperm chromatin. A more thorough study has shown that the primary organization of the core as well as MN_{180} particles is basically the same in the chromatin from Drosophila embryos, rat liver and sea urchin sperm. Also, the relative arrangement of core histones in core, MN_{170}, MN_{180}, MN_{190} particles remains essentially unchanged (manuscript in preparation). Therefore here, for the sake of simplicity, the histone octamer was located on

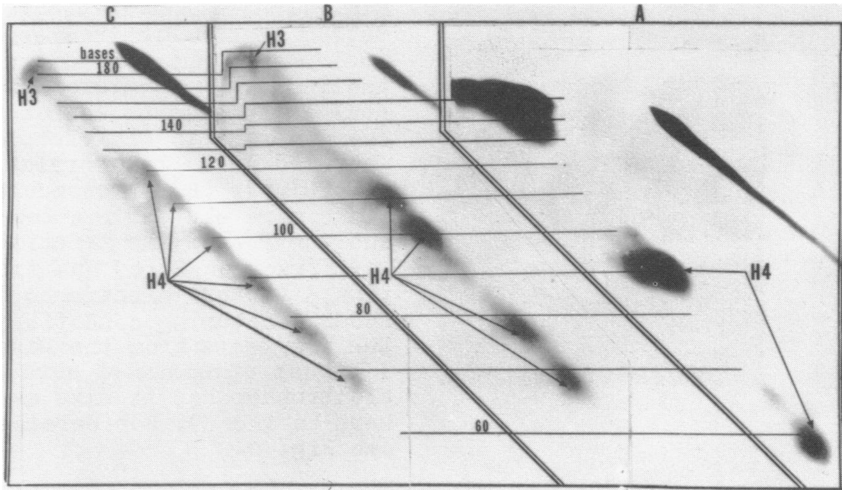

Fig. 4. Location of the binding sites for histones H4 and H3 on DNA in the sea urchin sperm core (A), MN_{180} (B) and MN_{190} (C) particles. The single-stranded 5'-terminal ^{32}P-labeled DNA fragments crosslinked to histones in nucleosomes were electrophoresed in 15% polyacrylamide gel from left to right. Then histones were digested with pronase, and the released ^{32}P DNA fragments were electrophoresed in 15% polyacrylamide gel in the second direction from top to bottom and identified by autoradiography (4). The DNA fragments produced by digestion of the nuclei with DNAase I were simultaneously electrophoresed in the second direction as size markers and identified by staining with ethidium bromide. Their length and position are indicated with figures and horizontal lines. The location of the DNA fragments crosslinked to histones H4 and H3 is marked with arrows.

nucleosomal DNA merely by taking account of the position of H4 histone spots in two-dimensional gels. Indeed, DNA fragments crosslinked to histone H4 are the most easily identified ones in the gels as intense and well-resolved spots located on a separate diagonal.

The DNA fragments crosslinked to histone H4 within the core particle give rise to two intensive spots of H4 (58) and H4 (90): figures in brackets show the size, in bases, of these fragments (Fig. 4A). In the gel for MN_{180}, each of these H4 spots in doubled and shifted by 10 or 20 bases upward: H4(58) becomes H4(68) and H4(78), whereas the spot for H4(90) is sub-

stituted by H4(100) and H4(110) (Fig. 4B). These data can most directly be explained by suggesting that the MN_{180} particle is an asymmetric one that can be formed from the symmetric core by extension of its 150 bp long DNA by 10 bp spacer from one end and by 20 bp spacer from the other (Fig. 5).

For the MN_{190} particle, the H4 spots are tripled and shifted up by 10, 20 and 30 bases: H4(58) are substituted for H4(68), H4(78) and H4(88), whereas H4(90) becomes H4(100), H4(110) and H4(120) (Fig. 4C). These data indicate that the MN_{190} particle is very probably a mixture of two different nucleosomes, viz. symmetric and asymmetric ones. A symmetric MN_{190} can be derived from the symmetric core by extension of its both DNA ends by 20 bp, while an asymmetric MN_{190} is formed upon the extension of the two core DNA ends by 10 and 30 bp spacers (Fig. 5). Fig. 5 also shows the structure of a symmetric MN_{170} particle observed in a mixture with MN_{180} /5/. An important feature of such a two-dimensional gel for MN_{180} particle isolated from Drosophila chromatin is the demonstration in this laboratory of crosslinking histone H3 to the terminal regions of the nucleosomal DNA (see Fig. 5 in ref.5). This strongly suggests that histone H3 interacts with the spacer DNA. H3 crosslinking to the terminal DNA regions can be seen less clearly for the MN_{180} and MN_{190} particles isolated from sea urchin sperm chromatin (Fig. 4).

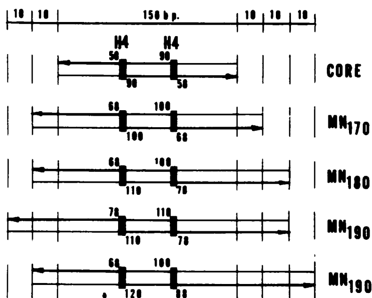

Fig. 5. Arrangement of the cores in mononucleosomes MN_{170}, MN_{180}, MN_{190}. In the symmetric core particle, two molecules of histone H4 are both crosslinked to the 5'-terminal DNA fragments 58 and 90 bases long. In MN_{180} and MN_{190}, the length of the H4-crosslinked fragments is increased by 10 and 20 or by 10, 20, and 30 bases of the spacers, respectively (Fig.4). The symmetric MN_{170} has already been described (5). The H4-crosslinked DNA fragments are shown by heavy lines; the arrows indicate the 5'-termini.

DISCUSSION

The approximately ten-base periodicity in the length of core and spacer DNA. The about ten-base regularity in the length of DNA fragments produced by digestion of chromatin from different sources with DNAase I /17, 18/, exonuclease III /25/, or *Serratia marcescens* nuclease /26/ extends well beyond the size of the nucleosomal repeat. Micrococcal nuclease also splits DNA in the core particles with such a regularity /27/. Therefore, it is not surprising to reveal a series of mono-, dinucleosomal and an intermediate-length DNAs in the micrococcal nuclease digests of *Drosophila* chromatin differing in size by about 10n bases and, in addition, to find out 20, 30, and 40 bp long spacers in dinucleosomes. We conclude from these results that the length of the spacer as well as of the core DNA is an integral multiple of the same 10 bp. Nuclease cutting sites in the core particle are spaced by approximately 10.4 bases /2/ but the spacing varies significantly from site to site /28/ and might be accomodated into both the 10.4 and 10.0 bp periodicity of the DNA duplex /2/.

The mono- and dinucleosomal DNA multiplicity and oligonucleosomal DNA heterogeneity is apparently caused by preferential and regular splitting of the intercore DNA with micrococcal nuclease at several 10 bp spaced sites. An example in Fig.6 schematically illustrates the appearance of a series of 6 fragments of MN_{160}-MN_{210} by random cutting two 30 bp spacers, flanking the core from the both sides, at 8 sites located at 10 bp intervals. The structure of a symmetric MN_{170}, an asymmetric MN_{180}, and a mixture of symmetric and asymmetric MN_{190} shown in Fig. 6 was directly determined while that of MN_{160}, MN_{200}, and MN_{210} is postulated.

Alignment of the cores in chromatin and the nucleosome phasing. The finding in Drosophila chromatin mainly of 20, 30, and 40 bp long spacers indicates that the chromatin may be composed from nucleosomes MN_{170}, MN_{180}, and/or MN_{190}. However, 180±5 bp long nucleosomal repeat and other data discussed below provide evidence that this chromatin consists of the regularly arranged repeating units, the MN_{180} particles, which

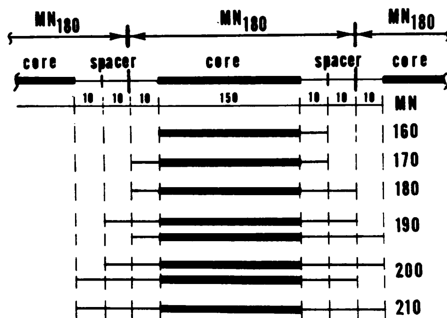

Fig. 6. A scheme explaining the multiplicity of mononucleosomal DNAs. It shows three adjacent asymmetric MN_{180} whose inter-core DNA has the same 30 bp. length. Random splitting of the spacers at regular sites located at 10 bp. intervals produces 6 mononucleosomal DNAs from MN_{160} to MN_{210}.

contain the DNA of the same length as the chromatin repeat.

In the asymmetric MN_{180}, the spacer has a length of 10 bp from one DNA end ('head') and 20 bp from the other ('tail'). In chromatin, the adjacent closely packed MN_{180} particles can be arranged along the DNA in three different orientation ('tail-to-head', 'tail-to-tail' and 'head-to-head') giving rise to 30, 40 and 20 bp long spacers, respectively (Fig. 7). It seems that all the three orientations of the adjacent MN_{180} particles occur in chromatin because these three spacers are found in dinucleosomes with a similar frequency. The random orientation of MN_{180} along DNA gives rise to all the observed MN1-MN7 (Fig. 1) particles including the MN7 (MN_{220}) particles of the maximal length which can be derived from the adjacent 'tail-to-head' and 'tail-to-tail' nucleosome junction (Fig. 7). The strictly regular, only 'tail-to-head' or 'tail-to-tail' and 'head-to-head', orientation of MN_{180} is unlikely since it

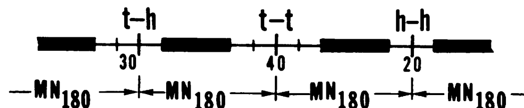

Fig. 7. A model for the alignment of MN_{180} particles in *Drosophila* chromatin. Chromatin is proposed to consist of the same MN_{180} that are regularly aligned and closely packed along DNA. The cores (black boxes) are placed asymmetrically in MN_{180} particles at a distance of either 10 bp. ('head', h) or 20 bp. ('tail', t) from the particle DNA edges. Three possible orientations of the adjacent asymmetric MN_{180} particles, namely 'tail-to-head' (t-h), 'tail-to-tail' (t-t) and 'head-to-head' (h-h), give rise to 30, 40 and 20 bp. long spacers respectively.

would lead only to 30 bp or 20 and 40 bp long spacers, respectively. Another model of the chromatin organization is a structure in which all of the three spacers are randomly distributed over chromatin. This model is however inconsistent with the regular arrangement of nucleosomes along chromatin. In addition, an MN_{230} particle would then appear from the cores flanked at the both sides with 40 bp long spacers which we have not observed.

These data suggest that chromatin in Drosophila consists of the regularly aligned, closely packed and randomly oriented MN_{180}. Within MN_{180} in chromatin, the core or symmetric MN_{170}, a chromatosome /29/, can occupy two alternative positions that are spaced by 10 bp. This is in agreement with the presence of several, shifted relatively each other by about 10 bp, frames for the cores in chromatin containing hsp70 Drosophila genes /11/ and rat liver satellite I DNA characterized by 185 bp nucleosomal repeat /10/.

This model of the chromatin structure can account for the origin of the nucleosome phasing which has also been found in Drosophila chromatin for hsp83 genes /9/ and histone genes /12/. The model seems to resolve the contradiction existing between the nucleosome phasing and heterogeneity of the spacer length /30/. According to the model, it is enough to determine the position of one 'specifying' nucleosome in Drosophila chromatin in order to align, in a precise way, all of the neighbouring closely packed MN_{180} particles whose random orientation is responsible for variations in the spacer length.

The organization of the spacer. It has recently been shown that core histones alone can organize and protect 168 bp against micrococcal nuclease digestion /31/. The DNA of this length appears to make two full turns of the superhelix in the MN_{170} particle, a chromatosome, in a way similar to that in the core particle /3, 32/. Two lines of evidence suggest that the entire 20–40 bp long spacer in Drosophila chromatin is also supercoiled through interaction with histone H3 as shown in Fig. 8 and form together with the core DNA the continuous superhelix. Firstly, the same ten-base periodicity in the location of nuclease cutting sites is equally applied for both

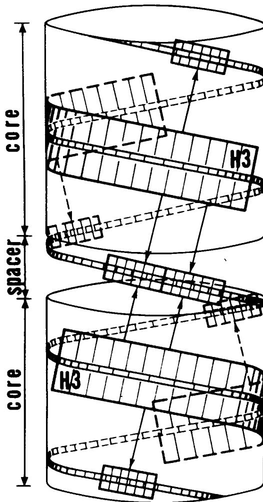

Fig. 8. A model for the superhelical organization of the spacer DNA by histone H3. The core and spacer DNA form a continuous left handed superhelix containing about 80 bp. per turn (3,32). The main body of H3 (wide boxes) interacts mainly with the central DNA region of the core (4). H3 also makes additional contacts (narrow boxes) in the direction of the arrows with the ends of the core DNA (4) and with the spacer (5). The middle core DNA region and the spacer are located side by side on the adjacent turn of the DNA superhelix.

the core and intercore DNA. Therefore, one may infere that the both DNA regions are exposed on the surface of the histone octamer /17/ and of the 10-nm chromatin fibril, and are organized in a similar, apparently superhelical, way. Secondly, the histone H3 was found to be crosslinked to the terminal, spacer and middle regions of DNA in the MN_{180} particle of Drosophila chromatin /5/ as well as in MN_{180} and MN_{140} of sea urchin sperm chromatin. This indicates that H3 molecules interact simultaneously with both of them and, by supercoiling, bring the ends of nucleosomal DNA close to its middle segment. Histone H3 and H1 were shown to crosslink to the same terminal regions of nucleosomal DNA /5/, location of H1 on these DNA regions has already been proposed /16, 19, 32/. It seems likely that histone H3 folds the spacer whereas H1 stabilizes /32/ the superhelical configuration of the intercore DNA. In the two adjacent core particles, their H3 molecules may interact with the intercore DNA with partial overlapping (Fig. 8), which could stabilize continuous supercoiling of the DNA over intercore regions. A model describing the path of DNA in chromatin as a continuous left-handed superhelix has already been proposed /33, 34/.

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