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The subunit structure of chromatin from Physarum polycephalum

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ABSTRACT

Nucleosome DNA repeat lengths in *Physarum* chromatin, determined by nuclease digestion experiments, are shorter than those observed in most mammalian chromatin and longer than those reported for chromatin of certain other lower eukaryotes. After digestion with staphylococcal nuclease for short periods of time an average repeat length of 190 base pairs is measured. After more extensive digestion an average repeat length of 172 base pairs is measured. Upon prolonged digestion DNA is degraded to an average monomer subunit length of 160 base pairs, with only a small amount of DNA found in lengths of 130 base pairs or smaller. Mathematical analysis of the data suggests that the *Physarum* nucleosome DNA repeat comprises a protected DNA segment of about 159 base pairs with a nuclease-accessible interconnecting segment which ranges from 13 to 31 base pairs. The spacing data are compatible with measurements from electron micrographs of *Physarum* chromatin.

INTRODUCTION

Studies on chromatin of several eukaryotic organisms have revealed the existence of a subunit structure consisting of repeating complexes of DNA and histones. Digestion of chromatin with nucleases allows the detailed analysis of DNA segments protected by proteins in this repeating subunit structure. Hewish and Burgoyne[1] demonstrated that an endogenous nuclease from rat liver cleaves nuclear DNA into a series of discrete segments which migrate on acrylamide gels as if they were multiples of a basic repeat. Subseqent digestion of chromatin of various species with exogenous staphylococcal nuclease has shown that the repeating unit of most chromatin contains an average DNA length of approximately 200 base pairs. Nuclease digestion studies complement other evidence obtained by electron microscopy[2-6], which revealed chromatin substructures resembling 'beads on string', X-ray diffraction[7-11], which demonstrated the presence of a repeating chromatin structure, and neutron scattering studies of the monomer chromatin product of nuclease digestion[12,13], which were consistent with a spheroid unit approximately 10 nm in diameter containing a histone core and

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DNA external to the core.

The subunit structure of chromatin from several eukaryotic species, including mammalian cell types[5,14] and the lower eukaryotes yeast[15], *Tetrahymena*[16], *Neurospora*[17] and *Aspergillus*[18] have been established. Some interspecies heterogeneity in sizes of repeating chromatin units has been noted. Lower eukaryotes frequently possess chromatin repeat lengths of DNA which are considerably shorter than the ∿ 200 base pair repeat length found in certain higher eukaryotes. Differences in chromatin subunit repeat lengths may be due in part to differences in histone composition among different classes of eukaryotes or to differences in the properties of histone H1.

The highly synchronous nuclear proliferative cycle of *Physarum polycephalum* and the availability of rDNA-containing chromatin from this organism[19,20] make it ideal for many studies on chromatin structure. Vogt and Braun[21] have recently reported that the length of DNA repeating units in *Physarum* chromatin is the same in metaphase and interphase, although precise measurements of repeat lengths are not given. We present here a detailed determination of sizes of *Physarum* chromatin structural components obtained after digestion with staphylococcal nuclease for different lengths of time. The results are discussed mathematically in terms of a chromatin model which assumes a regular arrangement of nuclease-resistant chromatin segments alternating with segments accessible to the enzyme, and which includes a term for time-dependent digestion of DNA 'tails' on nucleosomes.

EXPERIMENTAL PROCEDURES

Preparation of nuclei from *Physarum* microplasmodia and plasmodia.[34]

Physarum polycephalum (strain a x i) microplasmodia were grown at 25°C in 200 ml of culture medium[22] in shaking flasks as previously described[19]. To label DNA, microplasmodia were grown for 12 hr in medium containing ^{3}H-thymidine (5 μ Ci/ml; New England Nuclear). Microplasmodia from 3-day-old cultures were allowed to settle, washed 2 times with water at 4°, resuspended in 20 volumes of 0.32 M sucrose, 1 mM $CaCl_2$, 0.1% Triton X-100 and 0.1 M Tris-Cl, pH 7.2, and homogenized for 30 sec at high speed in a Waring blender. The homogenate was filtered through 1 layer of flannelette and centrifuged at 1000 x g for 10 min. The pellet was resuspended in 10 volumes of homogenization medium and rehomogenized for 10 sec in the Waring blender. The resulting nuclear pellet was resuspended in homogenization medium and recentrifuged as described.

Nuclease digestion of chromatin in *Physarum* nuclei.

Nuclei were suspended in 0.32 M sucrose containing 2 x 10^{-5} M $CaCl_2$ and 5 mM potassium phosphate buffer, pH 6.7 at a concentration of 4 x 10^8 nuclei per ml. *S. aureus* nuclease (P.-L. Laboratories, Milwaukee) was added to a final concentration 30 to 100 units of enzyme activity per ml of reaction mixture. Digestion was allowed to proceed at 37°C for varying lengths of time. The reaction was terminated by adding 1/5 volume of a solution containing 50 mM EDTA and 10% SDS. An equal volume of phenol, equilibriated with buffer containing 0.1% SDS, 10 mM EDTA, 0.1% 2-mercaptoethanol and 10 mM Tris-Cl, pH 8.0, was added to each digested sample. Protein was removed from digested chromatin samples by 3 successive phenol extractions followed by 1 extraction with an equal volume of chloroform. The aqueous phase following extractions was retained. DNA was precipitated from this aqueous solution upon addition of 3 volumes of ethanol and Na-acetate to a final concentration of 2% by standing for 12 hr at -20°. DNA precipitates were washed once with absolute ethanol and dissolved in buffer containing 10% glycerol, 8.3 mM EDTA and 0.03 M Tris-borate, pH 8.3, for electrophoresis.

Electrophoresis of DNA fragments.

Electrophoresis of DNA fragments from digested chromatin samples was conducted under non-denaturing conditions as described by Maniatis, et al[24], using 3.5% polyacrylamide gels containing 0.33% bis-acrylamide and electrophoresis buffer containing 2.5 mM EDTA and 0.01 M Tris-borate, pH 8.3. Gels were constructured as slabs 13 cm x 14 cm, 3 mm thick. Electrophoresis was performed for 3 hr at 100 volts. Marker dyes used were bromphenol blue (Sigma, St. Louis, Mo.) and xylene cyanol FF (George T. Gurr, Ltd., London). DNA in gels was stained by incubation of the slab in a solution containing ethidium bromide (Sigma, St.Louis, Mo.: 1 µg per ml) and 0.1 mM EDTA, pH 7.0, at 4° for 30 min.

Measurements of lengths of chromatin DNA segments.

Lengths of DNA segments from digested chromatin were determined by comparing electrophoretic mobilities of DNA digestion products with those of DNA sequences of known length. Two independent sets of DNA standards were used. Polyoma A2 restriction fragments were calculated from the data of Griffin, Fried and Cowie[25]. In some experiments λ dv-1 phage DNA digested with restriction endonuclease Bsu was employed as a standard set. The λ dv-1 restriction fragments have been sized by comparison of electrophoretic mobilities with those of sequenced segments of SV 40 DNA (R.E. Streeck and H.G. Zachau, personal communication). A direct comparison of the polyoma

A2 and λ dv-1 restriction digests, electrophoresed in parallel on a single slab gel is shown in Fig. 7. Independently-determined values for the restriction fragment lengths were in good agreement from 140 to 2200 base pairs (Fig. 7).

Electron microscopic visualization of Physarum chromatin subunit spacing.

Chromatin from growing *Physarum* microplasmodia was spread for electron microscopy by a modification of procedures previously described[26]. Microplasmodia were allowed to settle out of culture medium and were resuspended and sedimented 3 times at unit gravity from a solution of 1 mM ethyleneglycol-bis-(β-aminoethyl ether) N,N^1-tetraacetic acid (EGTA), pH 7.0. Washed microplasmodia were then resuspended in 300 volumes of a solution containing 0.5% Triton X-100 adjusted to pH 9.0 using 1 M sodium borate buffer, pH 10.0, and homogenized with 3 strokes of a Dounce homogenizer with a loose pestle. Aliquots of the *Physarum* homogenate were centrifuged onto carbon grids, strained with phosphotungstic acid and rinsed with ethanol exactly as described by Miller and Bakken[26]. Electron microscopy was performed using a Siemens 102 electron microscope.

RESULTS

Kinetics of chromatin digestion.

Physarum nuclei were digested with staphylococcal nuclease as described for varying lengths of time, and the percentage of DNA rendered TCA-soluble was determined, as shown in Fig. 1. It can be seen that about 60% of the chromatin DNA is solubilized upon extensive nuclease digestion. DNA fragments from nuclease-treated nuclei were purified and subjected to polyacrylamide gel electrophoresis in parallel with polyoma A2 viral DNA fragments of determined length, as shown in Fig. 2. It can be seen that prior to nuclease digestion, the nuclear DNA consists of high molecular-weight polynucleotides with low electrophoretic mobility in the gel. After 1 min of limited digestion under the conditions described, the resultant DNA segments migrate with a pattern characteristic of DNA lengths which are multiples of a basic repeating unit. After 60 min of nuclease digestion, virtually no DNA remains as large molecules detectable near the origin of the gel. Average numbers of nucleotide base pairs comprising each DNA fragment resolved have been determined by comparison with the depicted polyoma A2 standards as well as by comparison with Bsu restriction fragments from λ dv-1 phage DNA. Sizes of the chromatin DNA digestion products shown in Fig. 2 are presented in Table 1.

The data in Table 1 may be analyzed in terms of a chromatin model which

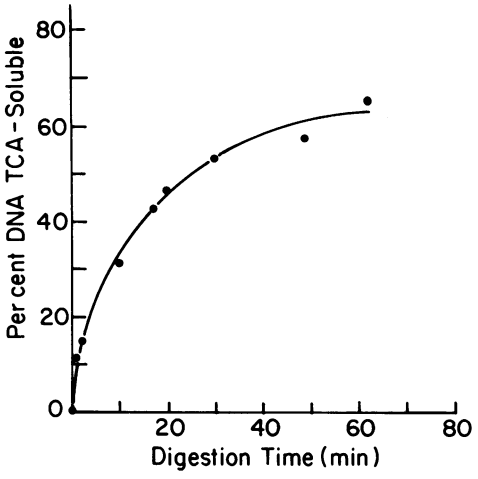

FIGURE 1 Time course of nuclease digestion of *Physarum* chromatin. *Physarum* nuclei (5 x 10^7/point; labeled as described with H-thymidine) were treated with staphylococcal nuclease (50 u g/ml) as described. The digestion reaction was terminated by addition of an equal volume of 10% TCA. TCA-insoluble material was collected by filtration Whatman GF-A filters, and assayed for radioactivity by scintillation spectrometry. Samples of the filtrate solution were also assayed for radioactivity. Note that about 60% of *Physarum* chromatin is rendered TCA soluble by nuclease treatment.

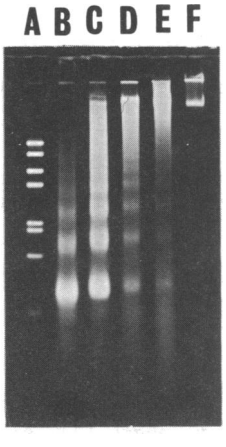

FIGURE 2 *Physarum* chromatin DNA digestion products. Chromatin in *Physarum* nuclei was digested with staphylococcal nuclease (50 u g/ml; 4 x 10 nuclei/ml) for different times at 37° as described in Experimental Procedures. Purified DNA (1.5 u g) from digested chromatin was subjected to electrophoresis on 3.5% polyacrylamide gels as described. A) Polyoma A2 DNA digested with restriction endonuclease Hpa II. B) 60 min digestion. C) 10 min digestion. D) 2 min digestion. E) 1 min digestion. F) DNA from nuclei prior to incubation with nuclease.

Table 1. Staphylococcal nuclease digestion of *Physarum* nuclear chromatin[a]

Digestion time t (min)	DNA band no. n	Distance migrated (cm)	Average fragment length ℓ (no. base pairs)[b]	Difference in lengths of successive oligomers (no. base pairs)	Average length of repeating unit $X_o + X_1$ (no. base pairs)[c]
1	0	8.46	179		190
	1	6.42	352	173	
	2	5.15	545	193	
	3	4.23	740	195	
	4	3.60	930	190	
	5	3.10	1120	190	
2	0	8.60	171		189
	1	6.49	344	173	
	2	5.22	532	188	
	3	4.30	725	193	
	4	3.64	920	195	
	5	3.15	1110	190	
10	0	8.67	167		180
	1	6.63	328	161	
	2	5.29	520	192	
	3	4.40	700	180	
	4	3.74	880	180	
60	0	8.81	160		172
	1	6.70	320	160	
	2	5.43	495	175	
	3	4.51	675	180	
	4	3.88	840	165	

[a] Digestion of *Physarum* nuclei was conducted as described for Fig. 2.
[b] Sizes of nuclear DNA fragments were measured by comparison with polyoma A2 DNA restriction fragments, as shown in Fig. 1. Sizes of restriction fragments used were obtained by direct comparison with λ dv-1 DNA digested with restriction endonuclease Bsu, as shown in Fig. 7.
[c] Repeat lengths were measured as the slope of a line, determined by the method of least squares, graphed through points for ℓ vs n at each digestion time.

assumes that chromatin prior to digestion consists of a DNA repeat of length $X_o + X_1$, in which X_1 is available for nuclease digestion and X_o is resistant (Fig. 3). After digestion every fragment on the gel will include at least one length X_o. Each fragment will also have two 'tails' from the X_1 regions. Staphylococcal nuclease contains both endonuclease and exonuclease activities. Thus the tails will be digested away, and the average length of each tail will be a function of digestion time, X_1 f(t). Therefore, every fragment will include at least one length X_o plus two tails, i.e., a length $X_o + 2X_1$ f(t) (Fig. 3). The longer fragments will also include undigested repeats, $n(X_o + X_1)$, where n = 0,1,2,3. . . according to the number of intact repeating units contained in the multimeric fragment. Thus, the total fragment length (ℓ) is:

$$\ell = X_o + 2X_1 \, f(t) + n(X_1 + X_o).$$

It can be seen from both Fig. 2 and Table 1 that the average DNA length in the monomer chromatin subunit produced ($\ell_{n=o}$) decreases as digestion time increases. The monomer length is about 179 base pairs after 1 min digestion and about 160 base pairs after 60 min digestion. It is possible to determine the length of nucleosome DNA resistant to nuclease digestion (X_o) using data

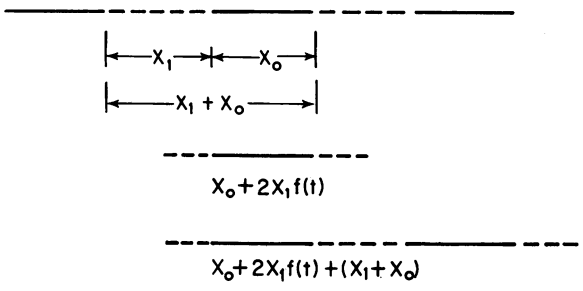

FIGURE 3 Alternating segments of nuclease-accessible and nuclease-inaccessible DNA in chromatin. X_1=length of nuclease-accessible segment. X_0= length of nuclease-inaccessible segment. t=digestion time.

presented in Table 1. If it is assumed that $f(t) \to o$ as $t \to \infty$ then a plot of ℓ vs $\frac{1}{t}$ for the monomer band (n = o), followed by extrapolation to $\frac{1}{t} = o$ gives X_o. Changes in the length of the monomer band with increasing digestion time are plotted in different ways in Fig. 4. In this case a plot of ℓ vs $\frac{1}{t}$ is inconvenient for extrapolation to $\frac{1}{t} = o$. A plot of ℓ vs $\sqrt{1/t}$ is somewhat more linear than ℓ vs $\frac{1}{t}$, and offers a better possibility of extrapolating to $\frac{1}{t} = o$ to estimate a value for X_o. A straight line obtained by linear regression has been drawn through points of the plot of ℓ vs $\sqrt{1/t}$ show in Fig. 4. The value of X_o thus estimated is 159 base pairs. The use of $\ell_{n=o}$ vs $\sqrt{1/t}$ for determining X_o is empirical, and the use of a straight line to represent this function is likely to be an oversimplification. Posible complexity in the variance of the monomer length with digestion time is indicated by the graph of $\ell_{n=o}$ vs $\frac{1}{t}$, as shown in Fig. 4.

The average length of DNA contained in the *Physarum* nucleosome repeat can be determined by measuring the slope of the line obtained upon plotting fragment length (ℓ) vs band number (n) at a given digestion time. Such lines, representing data presented in Table 1, are shown in Fig. 5. It can be seen that the plot of ℓ vs n is a straight line at each digestion time. The lines were analyzed by the least squares method, and each slope (X_o+X_1) is presented in Table 1. The length of DNA in the chromatin repeat obtained after 1 min. digestion is about 190 base pairs. With increasing digestion time the observed DNA repeat length is diminished, and after 60 min digestion, the average repeat length was found to be about 172 base pairs (Table 1). These values are in close agreement with the average repeat lengths determined from the difference between sizes of oligomers of (n + 1) and n repeats, where n > 3, as presented in Table 1.

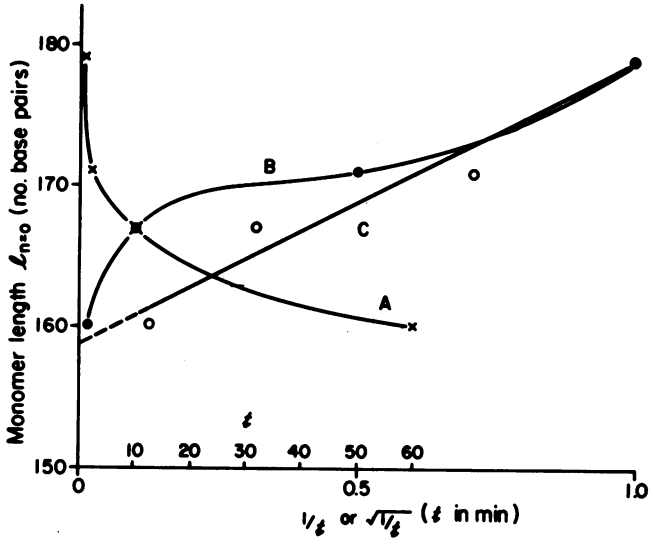

FIGURE 4 Kinetics of digestion of *Physarum* chromatin to monomer subunit length. $\ell_{n=o}$ = monomer DNA length. t = digestion time. Data plotted are those described in Fig. 2 and Table 1. A) $\ell_{n=o}$ vs t. B) $\ell_{n=o}$ vs 1/t. C) $\ell_{n=o}$ vs $\sqrt{1/t}$.

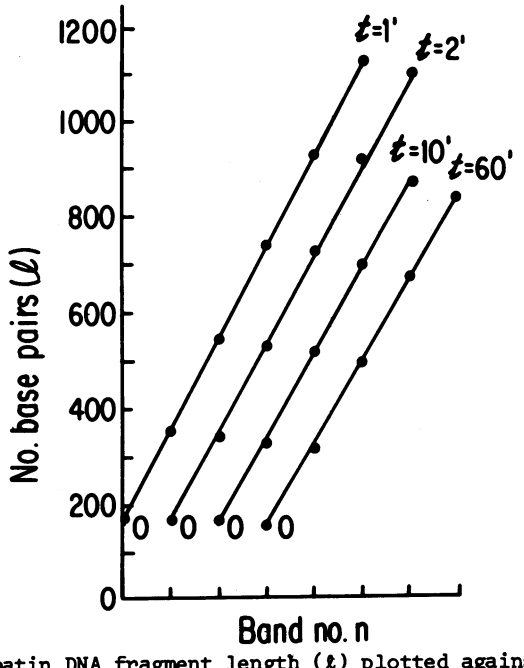

FIGURE 5 Chromatin DNA fragment length (ℓ) plotted against DNA band number (n) for acrylamide gel bands of DNA from nuclei digested for given lengths of time (t). Data plotted are those from Fig. 2 and Table 1. Plots for different given times are shifted one unit on the n axis to avoid overlap.

Comparison of nuclease digestion products from nuclei of rat liver and *Physarum*.

Rat liver nuclei were subjected to nuclease digestion for varying lengths of time, and gel electrophoretic mobilities of the resulting DNA fragments were compared with those of the polyoma A2 and λ dv-1 DNA standards as well as *Physarum* chromatin digests. The time course of conversion of chromatin DNA to segments representing multimeric repeats differed for rat and *Physarum*. Chromatin in rat nuclei was digested to monomeric subunits within 5 min using 100 units of nuclease per ml under the conditions described. 160 and 140 base pair units were produced. Approximately 10 min was required to digest *Physarum* chromatin to 160 base pair monomer units under similar conditions. In the liver nuclei there was no significant change in the nucleosome DNA repeat lengths observed upon digestion with nuclease for times between 30 sec and 2 min under the conditions described. Fig. 6 shows an acrylamide slab gel in which DNA from rat liver and *Physarum* nuclear digests have been electrophoresed in parallel. It is clear that in the rat and *Physarum* samples compared, the distances between rat multimeric DNA bands are greater than the corresponding

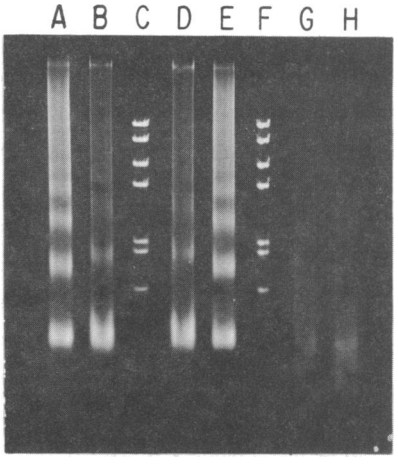

FIGURE 6 Comparison of electrophoretic patterns of rat liver and *Physarum* nuclear digests. Rat liver nuclei were digested with staphylococcal nuclease (100 units/ml; 1.8 x 10^8 nuclei/ml) for 2 min at 37° (B,D), and *Physarum* nuclei were digested (50 units of nuclease/ml; 2 x 10^8 nuclei/ml) for 10 min at 37° (A,E). *Physarum* nuclei were also digested for 10 min (G) or 20 min (H) with 100 units of nuclease/ml as described. Polyoma A2 DNA digested with restriction endonuclease Hpa II was electrophoresed as a standard set (C,F). Note that oligomeric DNA fragments from rat are longer than those from *Physarum*.

distances in the *Physarum* digest. Fig. 7 is a graph of a comparison of the sizes of rat and *Physarum* chromatin nuclease digestion products, as shown in Fig. 6, with polyoma A2 and λ dv-1 DNA standards. It can be seen that between 140 and 2200 base pairs there is good agreement between the two independently-sized sets of DNA standards, a comparison of which is shown in the inset to Fig. 7. Positions of DNA fragment bands from the rat liver 2 min nuclear digest and the *Physarum* 10 min nuclear digest are indicated on the standard line in Fig. 7. The rat nucleosome DNA repeat length, calculated as the slope of the ℓ vs n line, is about 201 base pairs. The *Physarum* repeat length, calculated similarly, is about 174 base pairs. When calculated by the method in which differences between adjacent multimer band sizes are averaged (Table 1), the rat repeat length is 201 ± 5 base pairs, and the *Physarum* repeat length is 173 ± 10 base pairs. Under no circumstances has a *Physarum* nucleosome DNA repeat length as large as 200 base pairs been observed, the largest repeat length, observed at 1 min of mild digestion, being about 190 base pairs (Table 1). Comparisons of *Physarum* chromatin DNA digests with digests of chromatin from nuclei of other mammalian cell types have also been conducted. *Physarum* repeat lengths obtained at all digestion times average less than corresponding repeat lengths obtained for nuclear DNA digests from HeLa cells and SK-L7 lymphocytes. Mammalian nucleosome DNA repeat lengths as low as 174 base pairs have not previously been reported.

After extensive nuclease digestion of nuclei, *Physarum* chromatin DNA is converted to a subunit monomer of average length of about 160 base pairs. Fig. 6 shows electrophoretic patterns of chromatin DNA digested for 10 min and 20 min with 120 μg/ml of staphylococcal nuclease. It can be seen that upon extensive digestion, the 160 base pair fragment is the primary DNA form, only a slight amount of DNA having been converted to DNA fragments of about 130 base pairs or smaller. This result is in contrast to results obtained upon extensive digestion of chromatin in mammalian nuclei. Chromatin DNA from rat liver, HeLa or SK-L7 cells is converted to fragments of 160 and 140 base pairs, and, upon further digestion, to fragments of 140 base pairs and smaller (data not shown). Note that the 130 base pair DNA fragment found in Fig. 1 does not decrease in size with nuclease treatment from 1 min to 60 min, as does the monomer DNA fragment. The basis for preservation of the 160 base pair monomer in *Physarum* nuclear digests is not known but may involve protection of a DNA segment by H1.

Electron microscopy of *Physarum* chromatin.

Chromatin from *Physarum* can be spread for electron microscopic

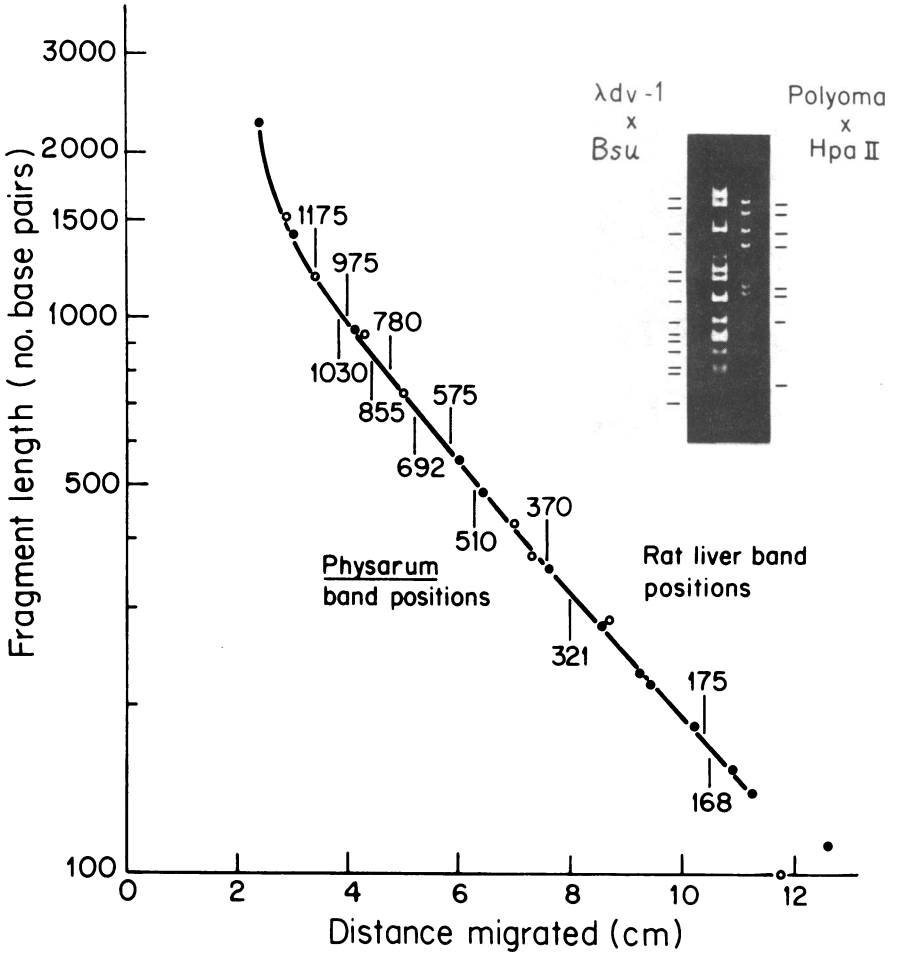

FIGURE 7 Sizes of rat liver and *Physarum* nuclear DNA digestion products. Rat and *Physarum* nuclei were digested for 2 min and 10 min, respectively, and DNA was subjected to electrophoresis on 3.5% polyacrylamide gels, as described in Experimental Procedures and in the legend to Fig. 6, along with polyoma A2 and λ dv-1 restriction fragment standards. Sizes of DNA segments in the 2 sets of DNA standards, independently determined, are plotted against migration distance, and a single curve has been drawn through the points. Open circles: polyoma A2 DNA digested with R. Hpa II. Closed circles: λ dv-1 DNA digested with R. Bsu. Note the agreement of the 2 standard sets between 140 and 2200 base pairs. Lines drawn to the bottom of the curve indicate average positions of *Physarum* chromatin digestion products. Lines drawn to the top of the curve indicate the position of rat liver chromatin digestion products. The inset shows a comparison of the 2 sets of DNA standards run in parallel on a single slab gel.

visualization using procedures employed for chromatin of various other organisms[3-6,26] with modifications intended to reduce slime contamination. Fig. 8 shows *Physarum* nuclear chromatin spread from growing microplasmodia.

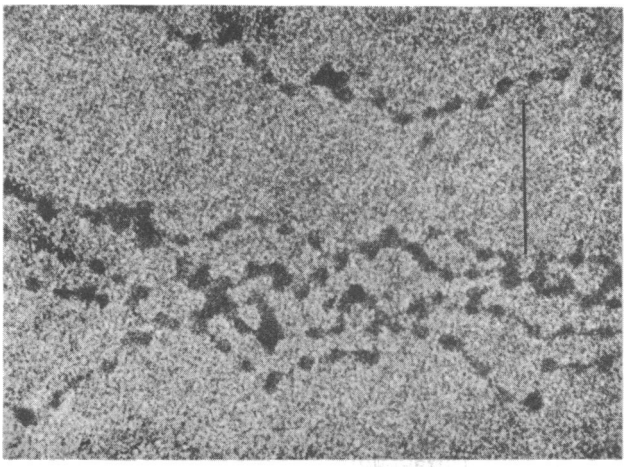

FIGURE 8 Electron micrograph of *Physarum* chromatin subunits. *Physarum* chromatin was spread for electron microscopy as described in Experimental Procedures. Chromatin was fixed with formalin and stained with phosphotungstic acid as described. The bar represents 0.1 μ.

This electron microscopic visualization confirms the subunit structure of *Physarum* chromatin evident on the basis of nuclease digestion studies. In the photograph shown, the size of nucleosome 'beads' averages 8.5 ± 1.4 nm in diameter. Interbead distance varies from no visible separation up to about 13 nm separation, and averages 7.5 ± 2.8 nm. Both bead size and interbead distance were found to be dependent upon spreading conditions employed. In those chromatin preparations in which interbead distance was greater than the average of 7.5 nm, the bead size was generally smaller than the average 8.5 nm. A total length of 16 nm based on photographic measurements from the centre of one bead to the centre of the next bead is an approximate average value for the *Physarum* chromatin nucleosome repeat length.

DISCUSSION

The data described indicate that nucleosome DNA repeat lengths, obtained upon digestion of *Physarum* nuclei for varying lengths of time, are shorter than repeat lengths obtained upon digestion of various mammalian nuclei. The *Physarum* nucleosome DNA repeat length of about 172 base pairs, obtained after 60 min digestion (Table 1) is similar to the nucleosome repeat length

of 170 ± 5 base pairs was recently reported for *Neurospora* chromatin DNA[17]. Chromatin of other lower eukaryotes has also been reported to consist of subunits of shorter DNA repeat lengths than those of mammalian chromatin. Repeat lengths of about 130 base pairs and 154 base pairs have been reported for yeast[15] and *Aspergillus*[18] chromatins, respectively. In contrast with these other lower eukaryotes, *Physarum* contains chromatin with DNA repeat lengths of up to 190 base pairs (Fig. 2, Table 1), and may, in this respect, be more similar to chromatin of higher organisms. Vogt and Braun[21] have obtained results indicating that *Physarum* chromatin repeat lengths are similar to those of mouse chromatin although no measurements of DNA fragment sizes were reported. It is possible that in their experiments digestion was conducted under conditions which revealed predominantly chromatin with longer DNA repeat lengths.

Our value of about 159 base pairs (Fig. 4) for a protected DNA region in the nucleosome repeat may be compared to recent results obtained by Noll and co-workers. These investigators reported that during digestion of rat chromatin an average monomer DNA length of about 160 base pairs is obtained which is eventually further digested to a smaller monomer unit of about 140 base pairs[29]. Chromatin depleted of histone H1 was found to be digested directly to a 140 base-pair monomer without pausing at 160 base pairs. In *Physarum* nuclear digests a DNA fragment of about 130 base pairs can be detected on acrylamide gels (Fig. 2), although no appreciable percentage of DNA is converted to fragments of this size even after extensive digestion. The *Physarum* nuclei used were found to possess a full complement of lysine-rich histone not easily removed by competition binding to tRNA (E.M. Johnson, unpublished observations). It is possible that protection by H1 of a DNA segment in the nucleosome repeat accounts for the predominant 160 base pair monomer obtained upon digestion of *Physarum* nuclei.

The apparent decrease in DNA repeat length with increased digestion time (Table 1) is a separate effect from the shortening of the 'tails', presumably by continued nuclease action on fragments, that has been observed by others[32,33]. The shortening of the 'tails' is accounted for by the term X_1 f(t) in the analysis and does not affect the calculation of repeat length. Several possibilities could account for the apparent decrease. One possibility is that *Physarum* nucleosome protected (X_o) DNA regions are connected by variable lengths of nuclease-accessible (X_1) regions. If longer X_1 regions are more susceptible to nuclease attack than are shorter X_1 regions, then at extended digestion times longer repeat lengths would be

degraded while shorter repeat lengths would remain to be detected. Assuming that the protected nucleosome DNA length in *Physarum* is constant at our calculated value of about 159 base pairs, it can be determined that the nuclease-accessible (X_1) connecting lengths vary from about 13 to 31 base pairs. Other factors besides variable X_1 regions could contribute to the observed decrease in nucleosome repeat lengths at longer digestion times. In particular the data do not distinguish between pre-existing variability in X_1 regions and variations that might arise during digestion by 'sliding' of protected X_o regions closer together as digestion proceeds. This putative 'sliding' could be facilitated by the action of endogenous enzymes such as proteases or histone modifying enzymes. The results obtained in the present investigation point out the importance of kinetic studies on chromatin digestion in experiments performed to measure sizes of chromatin DNA digestion products. Sizes of chromatin nuclease digestion products measured at a single digestion time may not accurately reveal the range of lengths of particular nucleosome regions present throughout the chromatin. Other workers have also observed a general decrease in oligomer DNA fragment lengths obtained at increased nuclear digestion times[15,27], although such decreases have not been related to changes in repeat lengths obtained at different digestion times. It is difficult to ascribe the progressive diminution in size of *Physarum* oligomer lengths to digestion of unprotected DNA tails at ends of segments. If such decreases were due solely to digestion of DNA at ends of oligomer units, it is likely that all oligomeric fragments would decrease in length at the same rate. Over the 60 min digestion period the pentamer, tetramer, trimer and dimer fragments decrease in size by 90, 65, 50 and 32 base pairs, respectively (see Table 1). The reason for this differential digestion of different oligomer chromatin units is not presently known, but may involve heterogeneity in the lengths of repeating units in *Physarum* chromatin DNA as noted above. Heterogeneity of calf thymus chromatin subunits has been suggested on the basis of studies of nuclease digestion products of submonomer length[28]. Recent observations of Garrard and Todd indicate the existence of different classes of nucleosome spacings in calf thymus chromatin (T. Garrard, personal communication). The possibility that *Physarum* contains different chromatin regions with different internucleosome spacings remains to be investigated.

Much of the data described supports the general notion[1,11,12,14,15,17,23,30] that staphylococcal nuclease cleaves at nuclease-accessible sites between protected DNA segments. A simplified model of a repeating chromatin

structure (Fig. 3) has been useful in analyzing data from nuclease digestion experiments. The linearity of plots of DNA fragment length (ℓ) vs band number (n) (Fig. 5) at a given digestion time (t) indicate that DNA fragment lengths are multimers of a basic repeat. Plots of monomer length $\ell_{n=o}$ vs $\frac{1}{t}$ and $\ell_{n=o}$ vs $\sqrt{1/t}$ allow an extrapolation to infinite digestion time to calculate a value for a protected DNA region (Fig. 4). The value of about 159 base pairs, calculated for this nuclease-resistant segment, is close to the observed value of the primary DNA fragment remaining upon extensive chromatin digestion (Fig. 7), and is close to a value of 160 base pairs observed as a DNA protected region in chromatin of other eukaryotes[29]. Thus, the data confirm the aspect of the model which suggests that DNA monomers observed at early digestion times consist of nuclease-resistant (X_o) segments associated with nuclease-accessible tails. The chromatin model described may be modified to account for further complexity. In particular, there may be partially protected DNA segments in chromatin which vary in their degree of accessibility to nuclease. In mammalian chromatin, such partial protection by H1 may account for the production of a monomer unit of about 160 base pairs before digestion to a core unit of about 140 base pairs[29].

The lengths of nuclease-accessible nucleosome interconnecting (X_1) segments have been calculated as ranging from 13 to 31 base pairs. This length estimate is compatible with spacing measurements from electron microscopic observations. In chromatin spread for visualization, an average internucleosome length of 7.5 ± 2.8 nm has been observed (Fig. 8). It is not presently clear whether such 'interbead' regions exist in native chromatin or whether they are the result of unfolding during spreading procedures. It is possible that 'interbead' distances seen after spreading correspond to nuclease-accessible chromatin lengths in nuclei. If this observed interconnecting strand consists primarily of DNA in the B helical form, its measured length corresponds to that expected for approximately 14 to 30 base pairs*. Studies on subunits located on DNA sequences of defined length should help in measuring precise distances between nucleosomes. Our data are consistent with those of Chambon[31] who has reported that in SV40 minichromosomes, about 50% of the nucleosomes are separated by DNA segments of irregular lengths which correspond to about 15% of the viral genome.

* This calculation is based upon a distance of 0.338 nm between DNA bases in the double helix B form[28].

Preliminary studies from this laboratory suggest that chromatin containing ribosomal DNA in *Physarum* is digested by staphylococcal nuclease to produce DNA fragments of submonomer length (130 base pairs or smaller) more rapidly than is the bulk of nuclear chromatin. It is of interest to determine whether nucleohistone complexes exist on ribosomal genes and spacers as they do on other regions of chromosomal DNA.

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REFERENCES

1. Hewish, D.R. and Burgoyne, A.L. (1973) Biochem. Biophys. Res. Comm. 52, 504-510.
2. Woodcock, C.L.F. (1973) J. Cell Biol. 59, 368a.
3. Olins, A.L. and Olins, D.E. (1973) J. Cell Biol. 59, 252a.
4. Finch, J.T., Noll, M. and Kornberg, R.D. (1975) Proc. Nat. Acad. Sci. USA 72, 3320-3322.
5. Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Cell 4, 281-300.
6. Woodcock, C.L.F., Safer, J.P. and Stanchfield, J.E. (1976) Exp. Cell Res. 97, 101-110.
7. Baldwin, J.P., Bosely, P.G., Bradbury, E.M. and Ibel, K. (1975) Nature 253, 245-249.
8. Wilkins, M.H.F., Zubay, G. and Wilson, H.R. (1959) J. Mol. Biol. 1, 179-185.
9. Pardon, J.F., Wilkins, M.H.F. and Richards, B.M. (1967) Nature 215, 508-510.
10. Bradbury, E.M., Molgaard, H.V., Stephens, R.M., Bolund, L.A. and Johns, E.W. (1972) Eur. J. Biochem. 31, 474-482.
11. Kornberg, R.D. (1974) Science 184, 868-871.
12. Bradbury, E.M., Hjelm, R.P., Carpenter, B.H., Baldwin, J.P. and Hancock, R. In: Molecular Biology of the Mammalian Genetic Apparatus, Elsevier-Exerpta Medica, North Holland, in press.
13. Pardon, J.F., Worcester, D.L., Wooley, J.C., Tatchell, K., Van Holde, K.E. and Richards, B.M. (1975) Nucleic Acids Research 2, 2163-2176.

14. Noll, M. (1974) Nature 251, 249-251.
15. Lohr, D. and Van Holde, K.E. (1975) Science 188, 165-166.
16. Gorovsky, M.A. and Keevert, J.B. (1975) Proc. Nat. Acad. Sci. USA 72, 3536-3540.
17. Noll, M. (1976) Cell 8, 349-355.
18. Morris, N.R. (1976) Cell 8, 357-363.
19. Bradbury, E.M., Matthews, H.R., McNaughton, J. and Molgaard, H.V. (1973) Biochim. Biophys. Acta 335, 19-29.
20. Molgaard, H., Matthews, H.R. and Bradbury, E.M. (1976) Eur. J. Biochem. 68, 541-549.
21. Vogt, V.M. and Braun, R. (1976) FEBS Letters 64, 190-192.
22. Daniel, J.W. and Baldwin, H.H. (1964) In: Methods in Cell Physiol, (D.M. Prescott, Ed.), Academic Press, New York, pp 9-41.
23. Arnott, S. (1976) In: Organization and Expression of Chromosomes (Report of the Dahlem Workshop), (V.G. Allfrey, E.K.F. Bautz, B.J. McCarthy and R.T. Schimke, Eds.) Berlin: Dahlem Konferenzen, in press.
24. Maniatis, T., Jeffrey, A. and van de Sande, H. (1975) Biochemistry 14, 3787-3794.
25. Griffin, B.E., Fried, M. and Cowie, A. (1974) Proc. Nat. Acad. Sci. USA 71, 2077-2081.
26. Miller, O.L., Jr. and Bakken, A.H. (1972) Acta Endocrinol. Suppl. 168, 155-177.
27. Sollner-Webb, B. and Felsenfeld, G. (1975) Biochemistry 14, 2915-2920.
28. Rill, R.L., Oosterhof, D.K., Hozier, J.C. and Nelson, D.A. (1975) Nucleic Acids Research 2, 1525-1538.
29. Noll, M. (1976) In: Organization and Expression of Chromosomes (Report of the Dahlem Conference Workshop), V.G. Allfrey, E.K.F. Bautz, B.J. McCarthy and R.T. Shimke, Eds.) Berlin: Dahlem Konferenzen, in press.
30. Axel, R., Melchior, W., Sollner-Webb, B. and Felsenfeld, G. (1974) Proc. Nat. Acad. Sci. 71, pp. 4101-4105.
31. Chambon, P. (1976) In: Organization and Expression of Chromosomes (Report of the Dahlem Workshop), (V.G. Allfrey, E.K.F. Bautz, B.J. McCarthy and R.T. Schimke, Eds.) Berlin: Dahlem Konferenzen, in press.
32. Shaw, B.R., Herman, T.M., Kovacic, R.T., Beaudreau, G.S. and Van Holde, K.E. (1976) Proc. Nat. Acad. Sci. USA 73, 505-509.
33. Greil, G., Igo-Kemenes, T. and Zachau, H.G. (1976) Nucleic Acid Res. in press.
34. Mohberg, J., and Rusch, H.P. (1971) Exp. Cell. Res. 66, 305-316