Proc. Natl. Acad. Sci. USA
Vol. 75, No. 2, pp. 705–709, February 1978
Biochemistry

Chromatin structure in the cellular slime mold *Dictyostelium discoideum*

(nucleosome/staphylococcal nuclease digestion/protease digestion/electron microscopy)

ANTONY C. BAKKE*, JUNG-RUNG WU, AND JAMES BONNER†

Division of Biology, California Institute of Technology, Pasadena, California 91125

Contributed by James Bonner, November 14, 1977

ABSTRACT **The structure of *Dictyostelium discoideum* chromatin has been studied by the following techniques: electron microscopy, staphylococcal nuclease digestion, acrylamide gel electrophoresis, sucrose gradient centrifugation, and melting. The basic unit of chromatin is the nucleosome, which is a particle 98.6 Å in diameter. Approximately 50% of the chromatin is protected from nuclease digestion, but this decreases when protease activity is not inhibited. The nucleosome contains 187 base pairs of DNA, including a 137-base-pair core and a 50-base-pair linker. The monomer nucleosome has an $s_{20,w}$ value of 11.5 S on isokinetic sucrose gradients. When the chromatin is melted, four transitions are observed, at 54.5°, 66.7°, 74.9°, and 79.7°. The structure of *Dictyostelium* chromatin is very similar to that seen in higher eukaryotes.**

Olins and Olins (1) described the basic unit of chromatin structure, or nucleosome [also called the ν (nu) body or ψ (psi) particle]. By carefully spreading the chromatin they saw 70-Å beads strung together in long chains, which were linked by thin, 15-Å, DNA-like threads. The appearance of the linker is somewhat unstable because it is determined by salt concentration (2, 3) and the presence of histone H1 (4).

Baldwin *et al.* (5) determined the position of the elements in the simple monomer nucleosome of calf thymus, using neutron diffraction. There is an inner protein core 64 Å in diameter, surrounded by a 20-Å-thick sheath of DNA, making the entire structure 104 Å in diameter. Kornberg and Thomas (6) suggested that the protein core consisted of two each of four histones (H2a, H2b, H3, and H4) on the basis of crosslinking experiments and x-ray patterns. D'Anna and Isenberg (7) demonstrated that the histones bind to each other as specific pairs in solution with the tightest binding between H3 and H4. These two formed a tetramer (i.e., homotypic) to which two molecules each of H2a and H2b bound less tightly. On the other hand, Weintraub *et al.* (8) found that a tetramer of one each of H2a, H2b, H3, and H4 (i.e., heterotypic) could exist in high salt without DNA, and Altenburger *et al.* (9) showed that DNase II cleaves chromatin at 100-base-pair (bp) intervals under certain ionic conditions. These observations suggested that the monomer might be divided into two equal parts.

In chromatin the DNA helix wraps around the histone core. The length of the monomeric DNA varies widely from 154 to 241 bp, depending on the organism and tissue (10). *Aspergillus* has a monomer length of 154 bp (11), while mammals have longer pieces of DNA varying from 188 to 207 bp (12). Chicken erthrocytes have a longer repeat length (212 bp) than chicken liver cells (200 bp) and this difference apparently is due to variations in the length of the linker, which is determined by the presence of H1 or H5 histone (13). The presence of a linker is revealed when monomers are extensively digested. This produces a stable core length of DNA, which is about 140 bp in all organisms that have been investigated. The DNA between the cores is the linker and is covered by either H1 or its equivalent (14). Histones more basic than H1, such as H5, may require longer linkers.

Lower eukaryotes, such as *Dictyostelium discoideum*, are well suited for studying chromatin structure and gene expression. Because the slime mold has a large fraction of template-active chromatin, any structural differences in the active and inactive chromatin should be observable. One aspect of chromatin structure is confirmed in the following study. *Dictyostelium* has a less basic histone H1 than higher eukaryotes and also has a shorter length of linker DNA in the nucleosome. In addition, the presence of four, instead of the usual five, histones does not affect the basic chromatin structure.

METHODS

Growth and Labeling of Cells. A variant of *Dictyostelium discoideum* (Ax 3) is grown in an axenic culture (15) on a rotary shaker. The DNA is labeled with [^{3}H]thymidine for the nuclease digestion studies. The cells are first grown to 1×10^7 cells per ml. They are harvested and washed by centrifugation. They are then suspended at 1×10^8 cells per ml in 20 mM KCl/2.5 mM $MgCl_2$/50 mM sodium phosphate buffer, pH 6.5/[^{3}H]-thymidine at 4 μCi/ml and shaken for 8 hr. At the end of this time they are transferred to a minimal growth medium containing, per liter, 10 g of glucose, 10 g of proteose peptone (Difco), 0.5 g of yeast extract, 5 mM phosphate buffer at pH 6.6, 0.02 mg of biotin, 0.005 mg of cyanocobalamine, 0.2 mg of folic acid, 0.4 mg of lipoic acid, 0.5 mg of riboflavin, and 0.5 mg of thiamine (16). The concentration of yeast extract is decreased to remove competing unlabeled thymidine. The vitamins are added to compensate for the low level of yeast extract. The cells are diluted 10-fold to 1×10^7 cells per ml in the minimal medium and shaken an additional 10 hr. They are then harvested for use.

Preparation of Nuclei for Electron Microscopy. Slime mold cells are suspended in 0.1 M sucrose/10 mM Tris·HCl at pH 7/0.4% Nonidet P-40 (NP-40, Shell) at 2×10^8 cells per ml and are lysed by shaking for 45 sec. The suspension is centrifuged at $800 \times g$ for 5 min. The supernatant is discarded and the pellet is gently resuspended in the buffer and centrifuged. The nuclei are resuspended and adjusted to a concentration of 0.5×10^8 nuclei per ml. They are diluted 100-fold to 500-fold in 1 mM ethylenediaminetetraacetic acid (EDTA) at pH 7.5 and allowed

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; bp, base pair; $PhMeSO_2F$, phenylmethylsulfonyl fluoride.

* Present address: Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Boulevard, La Jolla, CA 92037.

† To whom reprint requests should be addressed.

to swell for 15 min. Carbon-coated copper grids are placed in a small well (17). The sample is layered over a 0.1 M sucrose cushion in the well and covered with a coverslip. The nuclei are centrifuged at 2000 × *g* for 5 min. The grids are removed, rinsed with 1% Photoflo (Kodak), and stained with 0.5% uranyl acetate for 30 sec. The uranyl acetate is carefully washed way, using a clean set of forceps, and the grids are air-dried. Finally, the grids are rotary shadowed with platinum and palladium (80/20) and viewed in a Phillips 300 electron microscope. The particles seen in the 35-mm film are measured with a Hewlett-Packard digitizer.

Nuclease Digestion. Labeled nuclei are prepared by first lysing cells in 0.37 M sucrose/40 mM KCl/20 mM phosphate buffer at pH 7.5/0.5 mM $Mg(OAc)_2$/0.1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA)/1.5 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$)/0.5% NP-40 at 2×10^8 cells per ml. The nuclei are pelleted at 1500 × *g* for 5 min. They are homogenized in the same buffer but with 2.5 mM $Mg(OAc)_2$ and 0.2% NP-40 and centrifuged at 2500 × *g* for 7 min. The nuclei are then homogenized in a 1 M sucrose buffer without NP-40 and centrifuged at 5000 × *g* for 10 min. They are resuspended in this buffer before use on the same day. An aliquot is removed and pelleted at 5000 × *g* for 10 min. The nuclei are washed once in the nuclease shearing buffer (5 mM Tris OAc, pH 7.8/20 mM NH_4OAc/0.4 mM $CaCl_2$/0.2 mM EDTA) and resuspended in it at 10 alkaline A_{260} units/ml. (An A_{260} unit is the amount of material having an absorbance of 1 when dissolved in 1 ml and the path length is 1 cm.) The samples are then digested with staphylococcal nuclease (Worthington) under the conditions described in the figures. Small aliquots are removed at the designated times and precipitated in cold, 7% perchloric acid. The pellets are washed with perchloric acid again and the supernatants are pooled for liquid scintillation counting in Aquasol (Amersham/Searle). After all the aliquots are removed, the remaining sample is made 1% in sodium dodecyl sulfate and aliquots are removed to determine the total radioactivity. All vials contain an equivalent amount of sodium dodecyl sulfate.

Gel Electrophoresis. The DNA is extracted from digested nuclei with phenol and chloroform. It is then ethanol precipitated and dissolved in one-tenth strength gel buffer with 10% (vol/vol) glycerol. The gel buffer is 89 mM Tris/89 mM boric acid/2.5 mM EDTA. The gels are run according to Peacock and Dingman (18) with 2% acrylamide and 0.5% agarose, but at 100 V and without cooling.

Sucrose Gradient Centrifugation. Isokinetic sucrose gradients are formed according to Noll (19) and centrifuged in a Beckman SW 41 rotor at an average rpm of 33,400 for 12 hr. The gradient characteristics are as follows: top concentration, 5.1% sucrose (wt/vol); reservoir concentration, 29.84% (wt/vol); mixing volume, 9.82 ml; minimum radius, 7.785 cm; maximum radius, 14.55 cm; sample volume, 0.5 ml; temperature, 4°; and particle density, 1.45 g/cm^3. The gradients are pumped through an Isco flow cell and the A_{260} is recorded. The samples contain labeled, digested rat liver nuclei as marker nucleosomes. Fractions are collected and counted in Aquasol with a Beckman scintillation counter.

Melting Chromatin. Unlabeled chromatin is purified from nuclei prepared as above. The nuclei are further washed twice with 0.05 M NaCl/5 mM EDTA/5 mM EGTA/1 mM $PhMeSO_2F$, pH 8, and then twice with 10 mM Tris/0.1 mM EGTA/1 mM $PhMeSO_2F$, pH 8. The resulting chromatin is suspended in nuclease shearing buffer and lightly sheared with staphylococcal nuclease to solubilize it in the buffer. This supernatant is dialyzed against several changes of 0.25 mM EDTA, pH 8, and then melted in a Gilford 2400 spectrophotometer using a thermo-programmer and thermal cuvette. The data are analyzed by a computer and fit to Gaussian components (20).

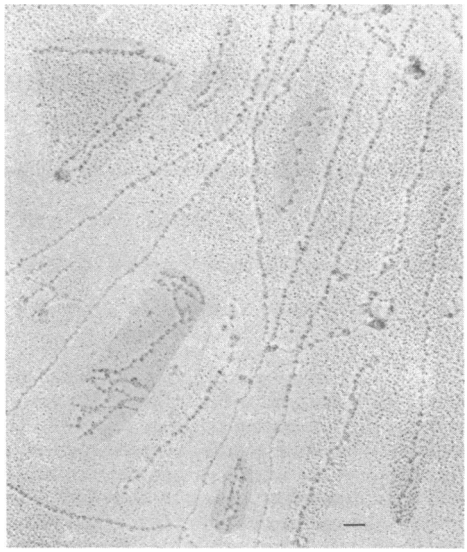

FIG. 1. A mosaic of several pictures of *Dictyostelium* chromatin taken with an electron microscope. The bar is 0.1 μm.

RESULTS

Electron Microscopy of *Dictyostelium* Nucleosomes. Nucleosomes may be observed by spreading them on grids as described in *Methods*. The solutions used must have a low ionic strength to allow swelling of the chromatin; otherwise it will remain condensed and nucleosomes will not be visible. In addition platinum–palladium shadowing greatly facilitates the microscopy by enlarging the nucleosomes and making them more dense. Fig. 1 is a mosaic of nucleosomes seen on several grids. They are found extending from nuclei, but the majority of the chromatin in the nucleus is still condensed. The nucleosomes are found in long strings and may be next to each other or with thin filaments connecting them. These filaments are assumed to be DNA. In unshadowed pictures the DNA filaments have an average diameter (±SD) of 30.9 ± 3.5 Å. The shadowed DNA has a diameter of 51.4 ± 9.3 Å. From x-ray crystallography work B form DNA is found to have a diameter of 20 Å (21). These figures are used to correct the measured diameters of the nucleosomes for the effects of preparation, yielding the corresponding real values. The diameters of all roughly circular particles are measured in several planes intersecting their centers and the averages are plotted as a histogram in Fig. 2. This distribution has an average and standard deviation of 98.6 ± 15.2 Å (n = 500) for the diameter of *Dictyostelium* nucleosomes. The length of the filaments between the nucleosomes is measured where they are present and the average is 170 Å or 50 bp. In the majority of cases no filament is visible. To further study the structure of chromatin, it is perturbed by the use of enzymes.

Nuclease Digestions of *Dictyostelium* Nuclei. Labeled, purified *Dictyostelium* nuclei are digested with staphylococcal nuclease for various lengths of time and the percent perchloric acid solubility is determined. Fig. 3 illustrates the results under several different digestion conditions. The nuclei are purified in the presence of $PhMeSO_2F$, a serine protease inhibitor, and, when $PhMeSO_2F$ is added to the digestion buffer along with the nuclease, acid-soluble DNA is released as shown in Fig. 3*A*. The digestion begins to plateau at 50%–55%, but there is a

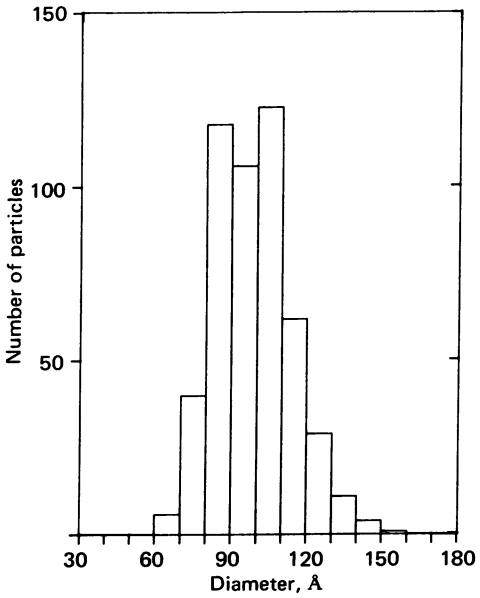

FIG. 2. A histogram of the corrected diameters of *Dictyostelium* nucleosomes (n = 500).

gradual increase to 70% between 30 and 60 min of digestion. If $PhMeSO_2F$ is removed from the nuclease buffer, digestion continues to 75% solubility as in Fig. 3*B*, indicating an increased sensitivity of the DNA. The relative initial rate of this reaction with respect to the control digest with $PhMeSO_2F$ (rate = 1.0) is 1.1. This is calculated by comparing the products of the first-order rate constant and the maximal percentage digested for the two reactions.

Because an endogenous protease could remove proteins and expose additional lengths of DNA to the nuclease, trypsin was added to mimic this activity. At a trypsin concentration of 10

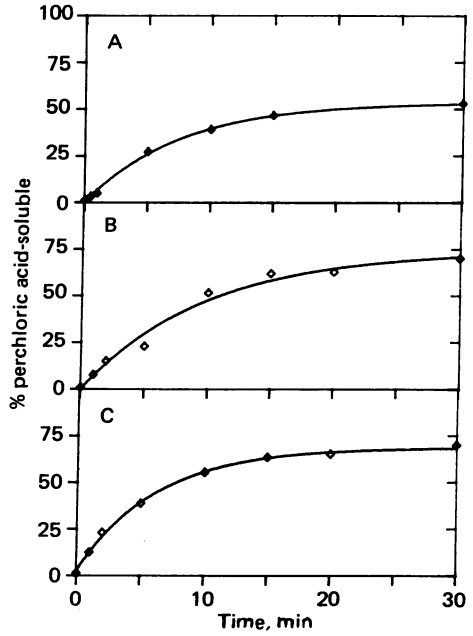

FIG. 3. Digestion of *Dictyostelium* nuclei with staphylococcal nuclease. (*A*) Nuclei are digested at 10 A_{260} units/ml (alkaline) and 100 units of nuclease per ml in the presence of 1 mM $PhMeSO_2F$. (*B*) Same conditions as *A*, except no $PhMeSO_2F$. (*C*) Nuclei are digested as above with trypsin added at 10 μg/ml and without $PhMeSO_2F$. (One unit of nuclease is that amount of enzyme which renders 1 μg of DNA acid soluble in 30 min at 37°.)

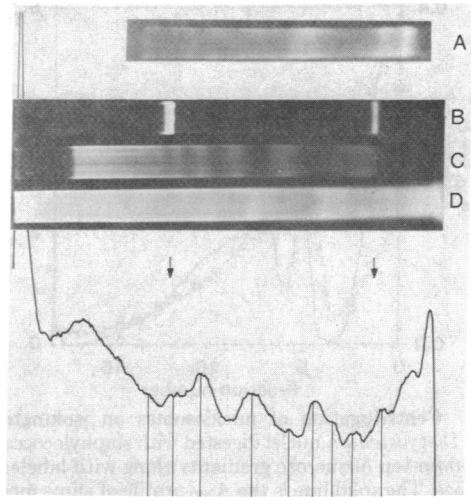

FIG. 4. DNA gels, 2% acrylamide with 0.5% agarose. Electrophoresis is from left to right. (Gel A) An early preparation of *Dictyostelium* nucleosomal DNA sheared with staphylococcal nuclease, which was electrophoresed with only rat liver nucleosomal DNA. It is approximately aligned with the other gels. (B) *Hin*dII restriction endonuclease fragments of φX174 DNA. The arrows mark the positions of fragments R1 (1049 bp) and R9 (161 bp). Nine markers are visible when the picture is overexposed. (C) Rat liver nuclear DNA sheared with staphylococcal nuclease. (D) *Dictyostelium* nuclear DNA sheared with staphylococcal nuclease. The optical scan is from the negative of gel D. The vertical lines mark the positions of the following lengths of DNA in bp from left to right: 1160, 967, 755, 483, 349, 275, 225, 184, and 136. Gels B, C, and D are adjacent lanes in one slab gel.

μg/ml and a DNA concentration of 350 μg/ml there is one trypsin molecule for every 1250 bp of DNA. The trypsin has an activity of 89.4 units/mg (22). The results of adding trypsin are shown in Fig. 3*C*. The acid solubility increases more rapidly, but it still plateaus at 75%. The relative initial rate with respect to the control digest is 1.6. When $PhMeSO_2F$ is added along with trypsin, the kinetics return to the original rate in Fig. 3*A*. The nuclei demonstrate a higher level of nuclease sensitivity after being stored for 8 hr at 0°, similar to Fig. 3*B*, even when $PhMeSO_2F$ is continually present.

Digestion may also be used to determine the length of the DNA in the nucleosome and the hydrodynamic size of the nucleosome. Nuclei are purified and digested with staphylococcal nuclease. Purified DNA is electrophoresed along with markers, which are used to prepare a standard curve for determining the size of the monomer repeat (Fig. 4). The monomer DNA size is measured by two methods: the length of the higher multimers divided by their multiplicity (12), and the difference in length between adjacent multimers (14). In the first case the length of monomer DNA is 187 ± 8 bp, and it is 190 bp in the latter. The nucleosome bands on gels are fairly broad, indicating some heterogeneity. However, some monomer DNA is apparently digested to a more constant, basic size, producing a sharper DNA band called the core (11). For slime mold it is 137 bp. The DNA that is removed from the monomer, leaving the core, is the linker DNA, which is generally covered by histone H1 or H5 (13, 14). Its length is determined either by measuring the half-width of a multimer band, or by finding the difference between the monomer and core length of DNA. This is 48 bp and 50 bp, respectively, in *Dictyostelium* and correlates well with the length of the filaments between nucleosomes seen in the electron microscope (50 bp). The DNA gels (Fig. 4) show some unusual complexity in the case of *Dictyostelium*. In the position of the putative dimer, a doublet is found with DNA

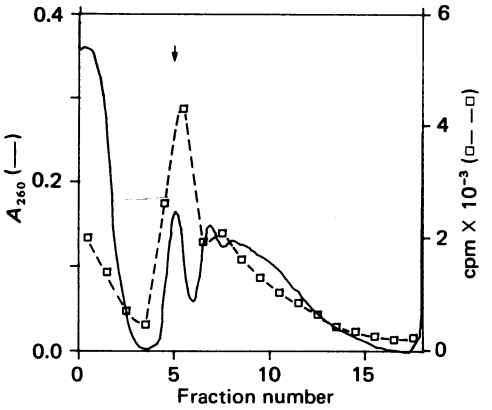

FIG. 5. Centrifugation of nucleosomes on isokinetic sucrose gradients. *Dictyostelium* nuclei digested with staphylococcal nuclease are layered on top of sucrose gradients along with labeled rat liver nucleosomes. The solid line is the A_{260} profile of slime mold nucleosomes. ^{14}C-Labeled rat liver nucleosomes are used as markers in the same gradient (□ - - □). The arrow marks the position of an 11.8S particle. Centrifugation is from left to right.

lengths of 225 and 275 bp. Below the doublet there are five additional peaks at 184, 137, 110, 96, and 78 bp.

The products of a nuclease digestion may also be analyzed on isokinetic sucrose gradients. In Fig. 5, slime mold chromatin is centrifuged along with labeled rat liver markers. The sedimentation coefficient ($s_{20,w}$) for rat liver nucleosomes is 11.8 S for the monomer and 16.3 S for the dimer. Slime mold monomers are not significantly different at 11.5 S, but the dimers are slightly smaller at 15.7 S. This agrees with the results from the gel electrophoresis, which indicate that *Dictyostelium* nucleosomes contain a shorter length of DNA.

Melting of *Dictyostelium* Chromatin. Basic proteins stabilize DNA against melting and the structure of chromatin may be elucidated by melting it (20, 23, 24). Slime mold chromatin is slowly melted in a low ionic strength buffer and the resulting data are differentiated in Fig. 6. Fig. 6*A* shows the derivative for pure *Dictyostelium* nuclear DNA. It is plotted in terms of change in hyperchromicity divided by change in Celsius degrees on the ordinate so that different melts may be compared. Fig. 6*B* is the equivalent derivative profile for slime mold chromatin, and four major Gaussian components may be fitted to this curve. The results of this fit and a comparison to a similar fit for rat liver chromatin are listed in Table 1. It is apparent that *Dictyostelium* chromatin also has four components, but in different proportions. The components may be compared between rat and slime mold by considering ones with similar differences between their t_m and the t_m of pure, homologous DNA. The lowest-melting component of *Dictyostelium* has a t_m only 16.0° higher than pure DNA, while the equivalent component in rat shows a difference of 21.2° in the t_ms. The second, third, and fourth components of both chromatins are similar with respect to their t_m differences, but they have different proportions. The second slime mold component is especially pronounced, while it is small in the rat. It seems that a larger fraction of the slime mold chromatin is poorly stabilized by its nucleoproteins.

DISCUSSION

Two conclusions may be drawn from this investigation of chromatin structure. First, the basic unit of structure in *Dictyostelium* chromatin is the nucleosome and its size is very similar to that found in higher eukaryotes. Second, endogenous proteases may drastically effect the chromatin structure. The

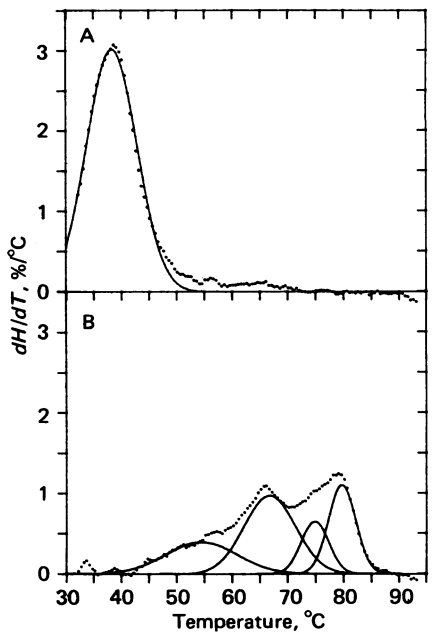

FIG. 6. Derivative profiles of melting curves for samples in 0.25 mM EDTA, pH 8. The plots are normalized to the same beginning absorbance. The ordinate scale has units of change in hyperchromicity per degree Celsius (dH/dT). (*A*) *Dictyostelium* nuclear DNA; total hyperchromicity is 36%. (*B*) *Dictyostelium* chromatin, total hyperchromicity is 29%. The dotted lines are the experimental values; the solid lines are the fitted Gaussian components.

measured diameter of the nucleosome is 98.6 Å and is very close to the 104 Å measured in calf thymus (5). Its core size of 137 bp has been found to be nearly identical in all organisms. These similarities are somewhat surprising in view of the fact that *Dictyostelium* has only four histones instead of the usual five, and one of the four is completely different. Apparently, the histones alone do not define the nucleosomes. This correlates with the results of Griffith (25), who found that under the correct salt concentrations free DNA would form nucleosome-like structures. Perhaps this aspect of the nature of the DNA is responsible for the uniformity in core size.

A DNA linker is attached to the core producing the monomer length of DNA and is associated with histone H1 (14). *Dictyostelium* has a shorter linker than higher eukaryotes, which may be a general characteristic of lower eukaryotes, in which H1 is less basic than in mammals (11, 26). The number of basic residues in the H1-equivalent molecule may define the length

Table 1. Summary of melting transitions from Fig. 6 and ref. 20

	Component 1		Component 2		Component 3		Component 4	
	t_m, °C	%	t_m, °C	%	t_m, °C	%	t_m, °C	%
			Dictyostelium					
Chromatin	54.4	22.6	66.7	39.7	74.9	14.0	79.7	23.7
DNA	38.4	100						
			Rat liver					
Chromatin	62.0	29.1	71.1	18.7	77.1	23.4	83.4	28.8
DNA	40.8	100						

Transitions are numbered in order of increasing temperature. Percentages refer to the fraction of the total hyperchromicity of each sample in a given transition.

of the linker. Histone H5 contains 37% basic residues and produces a linker of 72 bp in chicken erthyrocytes, while H1 has 30% basic residues and yields a 60-bp linker in chicken liver cells (27, 28, 13). *Dictyostelium* H1 is less basic than mammalian H1 (A. C. Bakke and J. Bonner, unpublished data) and the linker is only 50 bp long.

The visibility of the linker may also depend on H1. When H1 is present, the chromatin is condensed, with no visible DNA filaments connecting the nucleosomes. However, when H1 is removed, the fibers unfold into a string of beads separated by filaments (2–4). The conditions used in this study to spread the chromatin did not extract H1 and left most of the chromatin condensed with few visible linkers. This may partially explain the lack of monomer length DNA on the gels, while other conditions may give a more definite monomer band.

The nuclease digestion pattern is complex. A doublet of 225 and 275 bp is found at the position of the dimer. These are both small for a dimer with a 187 bp repeat, but may be explained by combinations of a core plus two linkers (~237 bp) and two adjacent cores with no linker (~276 bp). Endogenous protease or nuclease activity might also generate this pattern. The digestion studies emphasize the effect of proteases, which must be taken into account in interpreting data.

An alternate explanation of the digestion pattern is that the monomer repeat length is approximately 245 bp and the doublet represents the monomer and an intermediate degradation product. Because the presence of the linker allows some variability in the predicted results, the data could also fit a long repeat length. Lengths of 218 bp in sea urchin gastrula, 241 bp in sea urchin sperm, and 220 bp in *Stylonychia* macronucleus are reported in the literature (29, 30). However, fitting this long repeat size to the data for *Dictyostelium* could not explain the observed 190-bp differences in length between higher multimers or the 185-bp monomer length seen in earlier gels, which contain DNA prepared rapidly to reduce enzyme activity and are compared to rat liver nucleosome markers (Fig. 4, gel A).

A weight-average molecular weight, $\overline{M}_w$, of 245,000 for a nucleosome can be calculated using the Svedberg equation, a sedimentation coefficient of 11.5 S, a diffusion coefficient of 3.44×10^{-7} cm^2/sec, and an assumed density of 1.45 g/cm^3 (31, 32). It is very close to the predicted value for a combination of eight histones and 187 bp of DNA. With this $\overline{M}_w$ and the 98-Å diameter mentioned above, the nucleosome is apparently a disc 40 Å thick. This calculation is consistent with the disc-shaped nucleosomes observed by electron microscopy (33) and x-ray crystallography (34).

The melting of chromatin reveals additional details about its structure. *Dictyostelium* chromatin has four transitions, as seen in higher eukaryotes (20). The two at the highest temperatures are due to the melting of nucleosomes. The two highest transitions are both due to melting of histone–DNA complexes. The lower temperature transitions below these two are very broad and are due to the combined effects of histone H1 and basic nonhistone proteins. Because the digestion studies have shown that protease activity can quickly alter chromatin structure, any differences may be artifactual. Further studies must take this into account. However, it is still apparent that the chromatin is heterogeneous in its melting structure and that this fact may be used to dissect it. Further research may be able to correlate this with several observations: (*i*) the staphylococcal nuclease digestion pattern of *Dictyostelium* chromatin on gels is very heterogeneous; (*ii*) *Dictyostelium* contains a large fraction of expressed DNA (35); and (*iii*) *Dictyostelium* contains large ribosomal DNA units (36). Some of the heterogeneity in the melting transitions and in the DNA gel patterns may be due to the template-active and the ribosomal DNA.

We thank Dr. R. B. Wallace, T. D. Sargent, and J. W. Posakony for many valuable discussions. We also thank R. F. Murphy for the computer analysis of the data and Dr. Karyl Grohmann for the ϕX174 restriction fragments. This work was supported in part by U.S. Public Health Service Grant GM-13762, by U.S. Public Health Service Training Grant GM00082, and by a California Institute of Technology Fellowship to A.C.B.

1. Olins, A. L. & Olins, D. E. (1974) *Science* **183**, 330–332.
2. Renz, M., Nehls, P. & Hozier, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1879–1883.
3. Griffith, J. D. (1975) *Science* **187**, 1202–1203.
4. Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Cell* **4**, 281–300.
5. Baldwin, J. P., Boseley, P. G., Bradbury, E. M. & Ibel, K. (1975) *Nature* **253**, 245–249.
6. Kornberg, R. D. & Thomas, J. O. (1974) *Science* **184**, 865–868.
7. D'Anna, J. A., Jr. & Isenberg, I. (1974) *Biochemistry* **13**, 2098–2104.
8. Weintraub, H., Palter, K. & Van Lente, F. (1975) *Cell* **6**, 85–110.
9. Altenburger, W., Hörz, W. & Zachau, H. G. (1976) *Nature* **264**, 517–522.
10. Kornberg, R. D. (1977) *Annu. Rev. Biochem.* **46**, 931–954.
11. Morris, N. R. (1976) *Cell* **8**, 357–363.
12. Compton, J. L., Bellard, M. & Chambon, P. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4382–4386.
13. Morris, N. R. (1976) *Cell* **9**, 627–632.
14. Noll, M. & Kornberg, R. D. (1977) *J. Mol. Biol.* **109**, 393–404.
15. Cocucci, S. M. & Sussman, M. (1970) *J. Cell Biol.* **45**, 399–407.
16. Franke, J. & Kessin, R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2157–2161.
17. Miller, O. L., Jr. & Bakken, A. H. (1972) *Acta Endocrinol. (Copenhagen) Suppl.* **168**, 155–177.
18. Peacock, C. W. & Dingman, A. C. (1968) *Biochemistry* **7**, 668–674.
19. Noll, H. (1967) *Nature* **215**, 360–363.
20. Wallace, R. B., Sargent, T. D., Murphy, R. F. & Bonner, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3244–3248.
21. Watson, J. D. & Crick, F. H. C. (1953) *Nature* **171**, 737–738.
22. Hummel, B. C. W. (1959) *Can. J. Biochem. Physiol.* **37**, 1393–1399.
23. Li, H.-J. & Bonner, J. (1971) *Biochemistry* **10**, 1461–1470.
24. Shih, T. Y. & Bonner, J. (1970) *J. Mol. Biol.* **48**, 469–487.
25. Griffith, J. D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 563–567.
26. Noll, M. (1976) *Cell* **8**, 349–355.
27. Garel, A., Mazen, A., Champagne, M., Sautier, P., Kmiecik, D., Loy, O. & Biserte, G. (1975) *FEBS Lett.* **50**, 195–203.
28. Bustin, M. & Cole, D. R. (1968) *J. Biol. Chem.* **243**, 4500–4505.
29. Spadafora, C., Bellard, M., Compton, J. L. & Chambon, P. (1976) *FEBS Lett.* **69**, 281–285.
30. Lipps, H. J. & Morris, N. R. (1977) *Biochem. Biophys. Res. Commun.* **74**, 230–234.
31. Olins, A. L., Carlson, R. D., Weight, E. D. & Olins, D. E. (1976) *Prog. Nucleic Acid Res. Mol. Biol.* **3**, 3271–3291.
32. Brutlag, D., Schlehuber, C. & Bonner, J. (1969) *Biochemistry* **8**, 3214–3218.
33. Wooley, J. C. & Langmore, J. P. (1977) *J. Supramol. Struct. Suppl. 1*, abstr. 103.
34. Finch, J. T., Lutter, L. C., Rhodes, D., Brown, R. S., Rushton, B., Kevitt, M. & Klug, A. (1977) *Nature* **269**, 29–36.
35. Firtel, R. A. (1972) *J. Mol. Biol.* **66**, 363–377.
36. Cockburn, A. F., Newkirk, M. J. & Firtel, R. A. (1976) *Cell* **9**, 605–613.