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# Removal of Histone H1 Exposes a Fifty Base Pair DNA Segment between Nucleosomes†

James P. Whitlock, Jr.,* and Robert T. Simpson

ABSTRACT: Micrococcal nuclease has been used to prepare chromatin from HeLa cells and to probe the structure of HeLa chromatin under various ionic conditions and after the removal of chromatin proteins by salt extraction. The results suggest that (1) HeLa chromatin DNA exists as 150–160 base pair beads interspersed with 40–50 base pair bridges; (2) the bead and bridge conformation exists at physiologic salt concentrations; and (3) removal of histone H1 renders the 40–50 base pair bridge, but not the 150–160 base pair bead, more nuclease susceptible.

Morphological and biochemical evidence strongly supports the concept that chromatin has a repetitive structure, consisting of subunits composed of an octomeric core of the four small histones surrounded by a segment of the DNA fiber (reviewed by Felsenfeld, 1975). There is some disagreement as to whether all, or only a portion, of the DNA of the chromatin subunit is in close apposition to the histone core. Some investigators suggest that all the DNA of the chromatin subunit strongly interacts with the histone core to form a nucleoprotein "bead" (Noll et al., 1975; Finch et al., 1975); others suggest that only a portion of the DNA is in the "bead" conformation and that the remaining DNA, connecting neighboring subunits, is in a more extended and/or protein-free conformation, generating an overall "bead and bridge" structure (Olins and Olins, 1974; Shaw et al., 1974; Oudet et al., 1975; Langmore and Wooley, 1975; Simpson and Whitlock, 1976; Shaw et al., 1976; Woodcock et al., 1976). Electron microscopic observations (Griffith, 1975) suggest that, at low ionic strength ($I$ = 0.015), a bead and bridge structure might exist but, at higher salt concentrations ($I$ = 0.15), the beads become more closely apposed. We have shown previously that, at low ionic strength,

† From the Developmental Biochemistry Section, Laboratory of Nutrition and Endocrinology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014. *Received January 9, 1976.*

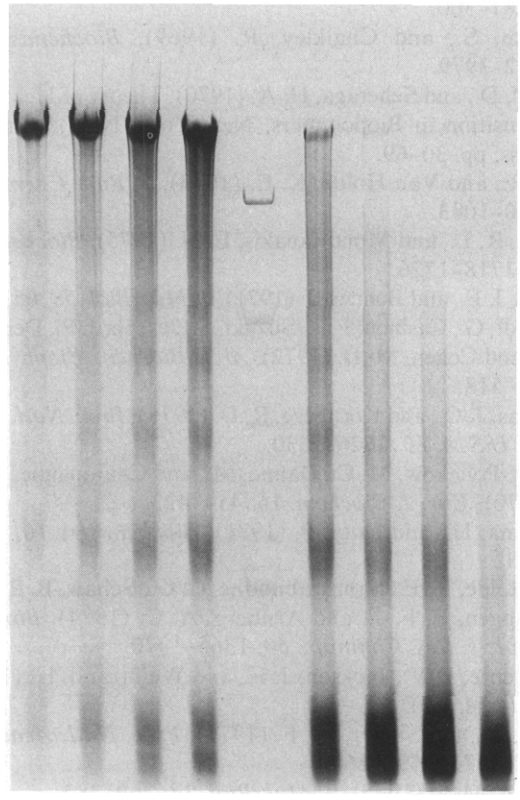

FIGURE 1: DNA fragments generated by nuclease digestion of nuclei. Nuclei were digested with micrococcal nuclease (100–1000 units/ml) for 10–120 min at 0 °C, and the DNA was purified as described in Methods and analyzed on 3% polyacrylamide gels. The middle slot contains the SV-40 Hae III digest standard DNA fragments. The remaining slots contain DNA from nuclei exposed to micrococcal nuclease for (left to right): 1000; 2000; 4000; 6000; 12 000; 24 000; 48 000; 120 000 units × min × $ml^{-1}$.

the DNA of the chromatin subunit contains at least two segments having different conformations. Nuclease-sensitive regions, 40–50 base pairs in length, are interspersed with relatively nuclease-resistant segments 150–160 base pairs long (Simpson and Whitlock, 1976). These chemical findings are analogous to the morphological observation that, at low ionic strength, chromatin has a bead and bridge structure.

A second problem in chromatin structure has been the location of histone H1. There has been disagreement over the presence or absence of H1 in preparations of the chromatin monomer. Baldwin et al. (1975) and Shaw et al. (1976) have suggested that H1 binds to the outside of chromatin particles.

We have studied the susceptibility of HeLa chromatin to digestion by micrococcal nuclease under various ionic conditions and after the removal of chromatin proteins by salt extraction. The results confirm our previous findings in rat liver suggesting that chromatin DNA has a bead and bridge structure. In addition, we find that chromatin has a bead and bridge structure at physiologic ionic conditions (0.15 M NaCl) and that histone H1 affects the nuclease susceptibility of the bridge segments of chromatin DNA.

## Material and Methods

*Cell Culture.* HeLa S-3 cells were maintained in logarithmic growth at 2–6 × $10^5$ cells/ml in Eagle's spinner medium No. 2 containing 5% horse serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Newly harvested cells were used in each experiment.

*Preparation of Nuclei.* Cells were collected by centrifugation, suspended in 5–10 volumes of ice-cold buffer A [0.25 M sucrose–3 mM $CaCl_2$–10 mM Tris-Cl[1] (pH 8)], made 1% in Triton X-100, and homogenized with 15 strokes of a loose-fitting glass Dounce homogenizer (Kontes, Vineland, N.J.). Nuclei were collected by centrifugation at 1600*g* for 1 min and washed once with buffer A without Triton X-100. Nuclei were washed twice with ice-cold digestion buffer [0.25 M sucrose–0.1 mM $CaCl_2$–1 mM Tris-Cl (pH 8)] and suspended at 20 $A_{260}$ units/ml for digestion with micrococcal nuclease (Worthington Biochemical Corp., Freehold, N.J.). Digestions were performed either in an ice–water bath or after warming the nuclear suspension to 37 °C.

*Preparation of Chromatin.* Chromatin was prepared from fresh nuclei using a modification of the method of Noll et al. (1975). Nuclei were suspended in digestion buffer at 35–40 $A_{260}$ units/ml, warmed in a 37 °C water bath for 4 min, and digested with micrococcal nuclease (15 units/ml) for 30 s. A $1/300$ volume of 0.1 M EDTA, pH 8, was added, and the suspension was chilled in an ice bath and centrifuged at 1600*g* for 1 min. The supernatant, containing 5–10% of the input $A_{260}$, was discarded. The pellet was gently suspended in ice-cold 0.2 mM EDTA, using a tight-fitting glass Dounce homogenizer, and allowed to swell for 30 min. The chromatin was diluted with 0.2 mM EDTA to 15 $A_{260}$ units/ml, and $1/300$ volume of 0.1 M $CaCl_2$ was added prior to digestion with micrococcal nuclease.

*Salt Extraction of Chromatin.* Chromatin was brought to the desired salt concentration by the slow addition of 5 M NaCl with swirling and centrifuged at 20 000*g* for 1 h at 4 °C. The supernatant, containing less than 10% of the input $A_{260}$, was discarded. The pellet was gently suspended in ice-cold buffer [0.1 mM $CaCl_2$–1 mM Tris-Cl (pH 8)] using a hand-held Potter-Elvehjem homogenizer with a Teflon pestle. The salt-extracted chromatin was suspended at a concentration of 15 $A_{260}$ units/ml for digestion with micrococcal nuclease.

*Analysis of DNA.* An aliquot of nuclei or chromatin was made 1% in sodium dodecyl sulfate, 10 mM in EDTA, and 0.1 M in Tris-Cl, pH 8, and extracted with an equal volume of buffer-saturated phenol for 30 min at room temperature. The aqueous phase was removed and made 0.1 M in NaCl, and the DNA was precipitated by the addition of 2 volumes of ethanol and storage overnight at −20 °C. DNA was collected by centrifugation, dissolved in electrophoresis buffer diluted fivefold with 10% sucrose, and analyzed on 3% polyacrylamide slab gels using the buffer system of Peacock and Dingman (1967). Gels were stained overnight in 0.005% "Stains-all" (Eastman) in 50% formamide (v/v) (Dahlberg et al., 1969), destained in running water, and photographed using Polaroid Type 55 P/N film. Negatives were scanned with an E-C Densitometer (E. C. Apparatus Company, St. Petersburg, Fla.). Double-stranded DNA standards for electrophoresis were fragments from an *Haemophilus aegyptus* restriction endonuclease (Hae III) digest of SV-40 DNA, having sizes of 1465, 820, 550, 370-300 (quintet), 220, and 165 base pairs (Lebowitz et al., 1974).

*Analysis of Proteins.* An aliquot of nuclei or chromatin was made 1% in sodium dodecyl sulfate, dialyzed overnight vs. 200 volumes of sample buffer, and analyzed in discontinuous sodium dodecyl sulfate–polyacrylamide gels using the buffers described by LeStourgeon and Rusch (1973). Histones were

[1] Abbreviation used: EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

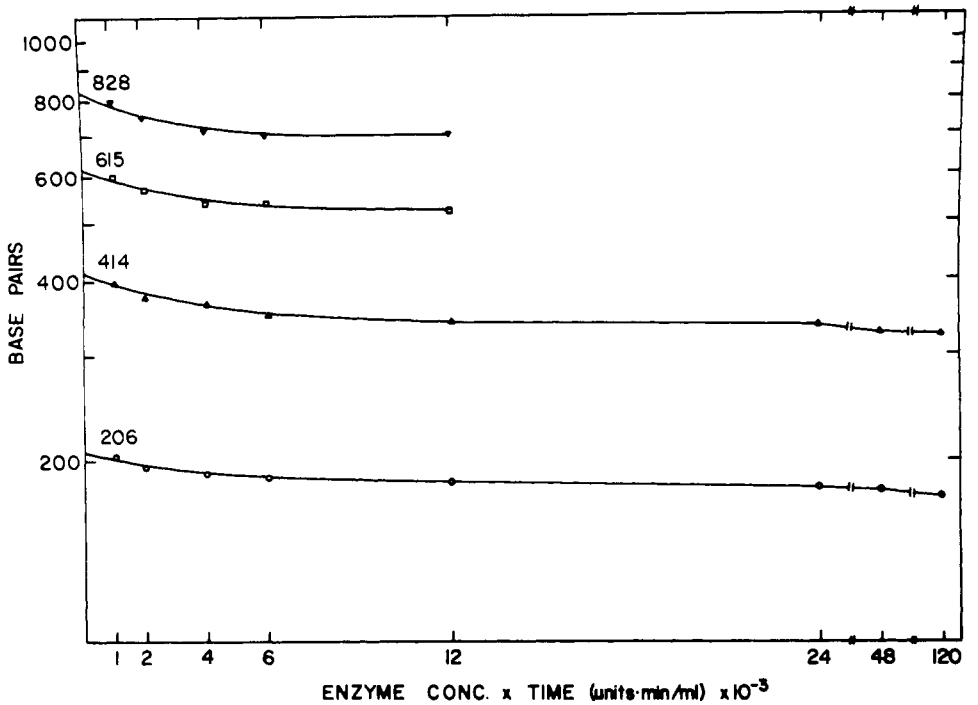


FIGURE 2: DNA fragment sizes as a function of amount of digestion. The sizes of the midpoints of the bands in Figure 1 were estimated by comparison with the standards. Extrapolation to zero nuclease digestion indicates that the initial cleavage occurs at intervals of 205–207 base pairs.

analyzed in 15% polyacrylamide gels containing an acrylamide–bisacrylamide ratio of 45:0.4. Nonhistone proteins were analyzed in 7.5% polyacrylamide gels containing an acrylamide–bisacrylamide ratio of 30:0.8. Stacking gels contained 3% acrylamide–0.08% bisacrylamide. Gels were stained for 1 h in 0.2% Coomassie blue (Sigma) in 50% methanol–7% acetic acid and destained in 20% methanol–7% acetic acid. Gels were photographed and the negatives scanned as described above for DNA. Protein concentration of chromatin was determined by the method of Lowry et al. (1951).

*Sucrose Gradient Sedimentation.* Aliquots of digested chromatin were layered over sucrose gradients containing 0.25 mM EDTA, pH 7, which were isokinetic for particles with a density of 1.51 at 4 °C. The meniscus concentration of sucrose was 5% (w/w). Gradients were formed as described by McCarty et al. (1974). Centrifugation was at 4 °C in the SW 41 rotor (Beckman) at 30 000 rpm for 16 h. Gradients were emptied by pumping from the bottom of the tube through a DB-G spectrophotometer equipped with a flow cell and log recorder.

## Results

*Digestion of Nuclei.* Brief digestion of HeLa cell nuclei by micrococcal nuclease under conditions of low ionic strength generates DNA fragments which migrate in polyacrylamide gels as multiples of a unit length (Figure 1). The typical pattern of bands appears when about 2% of the DNA has been made perchloric acid soluble; when 10–15% of the DNA has become acid soluble, all of the DNA in the gel migrates in such a pattern. Thus, HeLa cell nuclear DNA has a susceptibility to micrococcal nuclease similar to that of other tissues (Hewish and Burgoyne, 1973; Noll, 1974a; Axel, 1975; Sollner-Webb and Felsenfeld, 1975).

During the early stages of nuclease digestion, the "monomer" DNA band is composed of two distinct populations, one approximately 180–200 base pairs in length, the other about 150–160 base pairs (Figure 1); as digestion proceeds, most monomer fragments are converted to 150–160 base pairs. The results to be described suggest that the initial nucleolytic cleavage occurs at intervals of 205–207 base pairs, the length of the chromatin subunit DNA. The 150–160 base pair fragments represent the relatively nuclease-resistant DNA contained in the chromatin beads (nucleosomes). The remaining 40–50 base pairs of DNA of the chromatin subunit are in a more nuclease-sensitive conformation; these segments are analogous to the bridge regions observed by electron microscopy.

As digestion proceeds, the average length of the DNA fragments within each band decreases (Figure 1). Since this decrease is probably due to continued nuclease activity, we have extrapolated the average DNA lengths to zero nuclease digestion; this analysis suggests that the initial nucleolytic cleavage occurs at intervals of about 205–207 base pairs (Figure 2). The decrease in size of the fragments within each band occurs relatively early in the course of digestion; thereafter, the DNA lengths remain more stable. For example, the monomer band, initially containing DNA fragments about 200 base pairs long, decreases in size and then becomes stable at a DNA length of 150–160 base pairs. Similar observations in rat liver nuclei, combined with other evidence, suggest that the 200–205 base pair length of DNA in the chromatin subunit contains at least two segments having different conformations. First, there is a 40–50 base pair segment which is relatively susceptible to nuclease digestion and presumably is in a relatively extended and/or protein-free conformation; second, the remaining 150–160 base pair segment is relatively resistant to nuclease digestion and is more tightly complexed with nuclear proteins (Simpson and Whitlock, 1976). These chemical findings are analogous to the morphological observations of Griffith (1975) suggesting that the SV-40 minichromosome exists in a bead and bridge structure.

*Digestion of Chromatin.* Most investigations involving nuclease digestion of nuclei or chromatin have been performed at low ionic strength, both to maximize the solubility of the

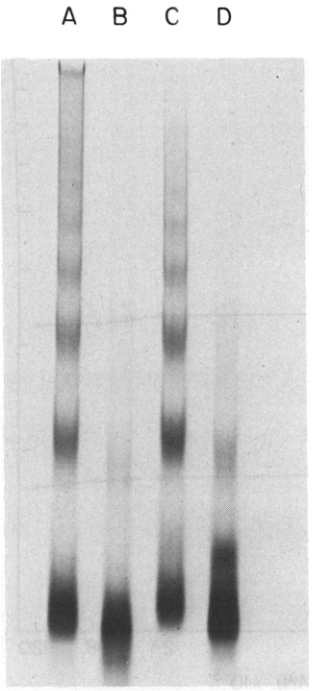


FIGURE 3: DNA fragments generated by nuclease digestion of chromatin without or with 0.15 M NaCl. Chromatin was prepared as described in Methods and digested with micrococcal nuclease (100 units/ml) for 16 or 64 min at 37 °C at different salt concentrations. The DNA was extracted and analyzed on 3% polyacrylamide gels. (A) No salt, 16-min digestion; (B) no salt, 64-min digestion; (C) 0.15 M NaCl, 16-min digestion; (D) 0.15 M NaCl, 64-min digestion.

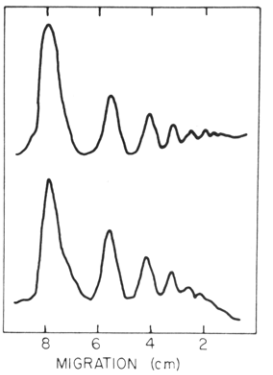


FIGURE 4: Densitometer tracing of the negative of a photograph of the gel in Figure 3. The upper tracing is of slot A, Figure 3. The lower tracing is of slot C, Figure 3. The ordinate is linear with optical density.

chromatin and to minimize the dissociation of nuclear proteins from the DNA. In the intact cell, however, the nucleoprotein is presumably exposed to a considerably higher ion concentration. Therefore, we have investigated the effect of increasing the salt concentration on the pattern of DNA fragments generated by nuclease digestion. We have elected to use chromatin, rather than nuclei, because of difficulties encountered in maintaining nuclei in a relatively homogeneous suspension at the higher salt concentrations.

Figure 3 shows typical examples of the DNA bands generated when chromatin is digested either under low salt conditions or in the presence of 0.15 M NaCl. Similar patterns of DNA fragments are generated in both ionic conditions and, in both instances, as digestion proceeds, the average size of the monomer band decreases. Thus, in general, the DNA in these chromatin preparations retains the susceptibility to nuclease digestion observed in intact nuclei. Again, digestion in 0.15 M NaCl generates a monomer band composed of two distinct populations of DNA fragments, one 180–200 base pairs in length, the other 150–160 base pairs. Later experiments explore this finding in more detail. Densitometer tracings (Figure 4) of the negatives of gel photographs reveal symmetric peaks and a low background, confirming that the chromatin is native, according to a criterion suggested by Noll et al. (1975). These results indicate that, in an ionic environment of 0.15 M NaCl, chromatin DNA still contains the relatively nuclease-sensitive and nuclease-resistant regions found in nuclear DNA under conditions of low ionic strength. This observation suggests that, at physiologic salt concentrations, chromatin DNA retains a bead and bridge conformation.

*Digestion of Protein-Depleted Chromatin.* In order to study the role of nonhistone proteins and H1 in the maintainance of chromatin DNA in a native conformation, we have exposed chromatin to increasing concentrations of sodium chloride, separated the chromatin from the dissociated proteins by centrifugation, and digested the remaining nucleoprotein under conditions of low ionic strength. Figure 5 indicates that chromatin which has been exposed only to low salt conditions, centrifuged, and resuspended in low salt buffer retains its native susceptibility to digestion by micrococcal nuclease. The removal of increasing amounts of chromatin proteins is associated with progressive changes in both the rate of digestion and the pattern of DNA fragments generated during digestion. First, there is a progressive increase in the rate of digestion, as measured by the generation of perchloric acid soluble products. The initial rate of digestion of chromatin which has been extracted with 0.6 M NaCl is about five times the rate of digestion of control chromatin (Figure 6). Both control and protein-depleted chromatin reach a limit digest when 50–60% of the DNA has been made acid soluble (data not shown). Second, after removal of chromatin proteins, the bands containing DNA fragments of multimeric size become progressively less well resolved even after brief digestions. Third, the monomer DNA band, 180–200 base pairs in length in native chromatin during early stages of digestion, undergoes a progressive decrease in size; after removal of the chromatin proteins soluble in 0.15 M NaCl, the monomer DNA band exhibits a shoulder on the faster migrating side. Following the removal of more protein, this shoulder becomes a discrete peak and finally becomes the major component of the monomer band after extraction of chromatin by 0.6 M NaCl (Figure 5). This decrease in the average size of the monomer band is similar to the decrease noted previously during the course of nuclease digestion of intact nuclei or chromatin. However, in these later experiments, the chromatin samples have each been digested an equivalent amount (as measured by the production of acid-soluble material); thus the decrease in the size of the monomer is a function of the removal of chromatin proteins, and not a function of the amount of nuclease digestion. The average size of the monomer DNA fragments generated by digestion of chromatin which had been extracted with 0.6 M NaCl is 150–160 base pairs; this is identical with the length of the relatively nuclease-resistant segment of the chromatin subunit. The results suggest that the removal of proteins by salt extraction increases the digestibility of 40–50 base pair segment of DNA within the chromatin subunit. Since the remaining 150–160 base pair DNA segment retains its relative resistance to nuclease digestion, it is likely that this 40–50 base pair DNA segment is the relatively nuclease-sensitive region of the chromatin subunit. Thus, the removal of chromatin proteins by 0.6 M NaCl affects primarily the properties of the bridge region of the chromatin subunit DNA.

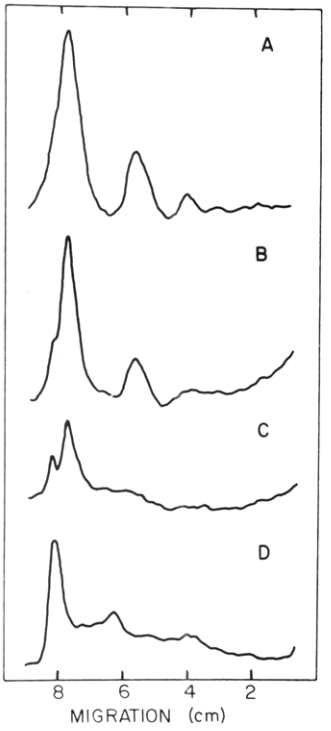


FIGURE 5: DNA fragments generated by nuclease digestion of salt-extracted chromatin. Chromatin was extracted with 0.20 mM EDTA plus (A) no salt, (B) 0.15 M NaCl, (C) 0.03 M NaCl, (D) 0.60 M NaCl, resuspended in 0.1 mM $CaCl_2$–1 mM Tris (pH 8) and digested with micrococcal nuclease (200 units/ml) at 37 ° C for (A) 4 min, (B) 2 min, (C) 1 min, (D) 1 min. The DNA was extracted and analyzed on a 3% polyacrylamide gel. The gel was photographed and the negative was scanned. The ordinate is linear with optical density.

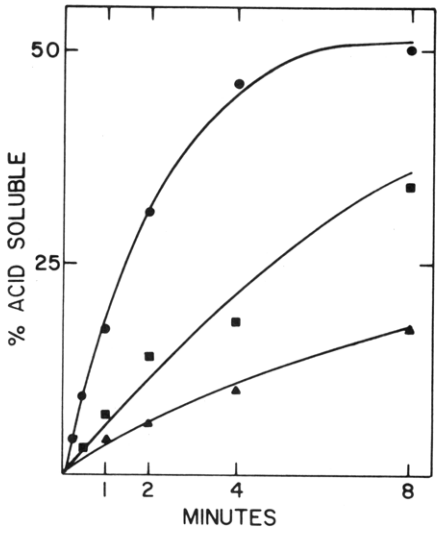


FIGURE 6: Kinetics of digestion of protein-depleted chromatin. Chromatin was extracted with 0.20 mM EDTA plus no salt (▲), 0.15 M NaCl(■), or 0.60 M NaCl (●) as described in Methods and then digested with micrococcal nuclease (200 units/ml) at 37 °C for the times indicated on the abcissa. The $A_{260}$ of the fraction soluble in 1% sodium dodecyl sulfate–0.6 M perchloric acid was measured.

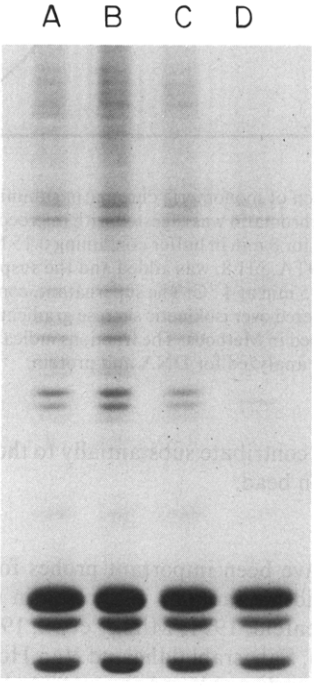


FIGURE 7: Proteins of salt-extracted chromatin. Chromatin was extracted with 0.20 mM EDTA plus (A) no salt, (B) 0.15 M NaCl, (C) 0.30 M NaCl, (D) 0.60 M NaCl and resuspended, and the chromatin proteins were extracted and analyzed in discontinuous 15% polyacrylamide–sodium dodecyl sulfate gels. The amount of protein applied to the gel was (A) 18 μg, (B) 24 μg, (C) 20 μg, (D) 14 μg.

Chromatin which has been extracted with 0.6 M NaCl has about 60% of the protein content of the original chromatin (data not shown). Electrophoresis in discontinuous 15% polyacrylamide–sodium dodecyl sulfate gels reveals that there is a progressive loss of histone H1 and nonhistone proteins as chromatin is exposed to increasing salt concentrations (Figure 7). The concentration of the four smaller histones is not substantially altered by the salt extractions. These results suggest that histone H1 and/or nonhistone proteins affect the conformation primarily of the 40–50 base pair bridge segment of the chromatin subunit DNA. As these proteins are removed, these DNA regions become even more nuclease-sensitive, resulting not only in the more rapid production of monomer subunits at the expense of multimeric subunits, but also in the more rapid digestion of the bridge DNA of each monomer, leaving the relatively nuclease-resistant bead regions intact.

These results were confirmed by isolating from an isokinetic sucrose gradient chromatin monomer subunits either containing or lacking the bridge DNA region (Figure 8). Electrophoresis in 3% polyacrylamide gels reveals that the DNA isolated from the peak of the gradient is 150–160 base pairs in length; however, the DNA isolated from a shoulder on the leading edge of the peak contains not only 150–160 base pair fragments but also a smaller amount of 180–200 base pair fragments (Figure 9). Analysis of the proteins isolated from these two regions of the sucrose gradient reveals virtually no histone H1 associated with the monomer fraction containing only 150–160 base pair DNA and a small amount of H1 in the fraction containing the 180–200 base pair fragments (Figure 10).

The removal from chromatin of proteins soluble in 0.6 M NaCl apparently does not substantially alter the conformation of the bead region of the chromatin subunit. The pattern of DNA fragments less than 160 base pairs in a limit digest of chromatin extracted with 0.6 M NaCl is similar to the pattern of fragments in a limit digest of control chromatin (Figure 11; Axel et al., 1974; Weintraub and Van Lente, 1974). The fragments in a limit digest are thought to reflect histone–DNA interactions within the chromatin subunit. Our results suggest that histone H1 and/or nonhistone proteins extractable in 0.6

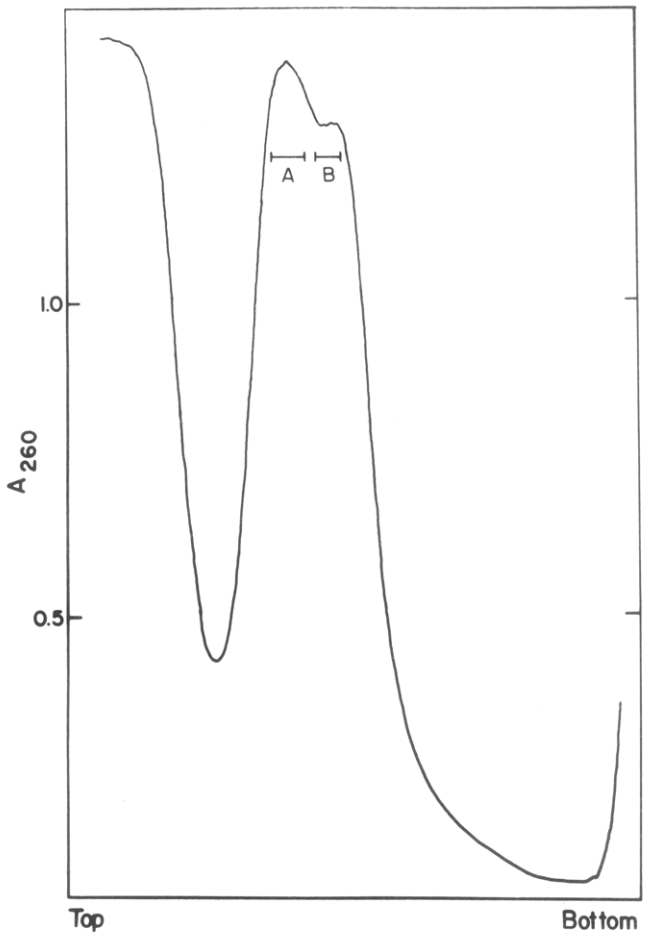


FIGURE 8: Isolation of monomeric chromatin subunits on an isokinetic sucrose gradient. Chromatin was digested with micrococcal nuclease (200 units/ml) at 37 °C for 8 min in buffer containing 0.15 M NaCl. A volume ($1/300$) of 0.1 M EDTA, pH 8, was added and the suspension was centrifuged at 6000*g* for 5 min at 4 °C. The supernatant, containing 30% of the input $A_{260}$, was layered over isokinetic sucrose gradients, centrifuged, and analyzed as described in Methods. The fractions indicated by the brackets were collected and analyzed for DNA and protein.

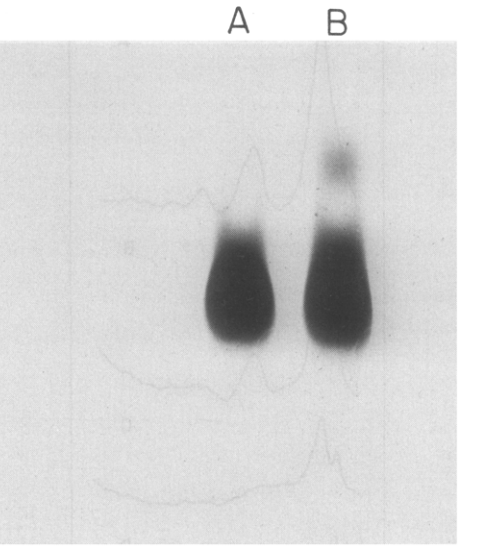


FIGURE 9: DNA was extracted from the fractions indicated in Figure 8 and analyzed on a 3% polyacrylamide gel.

M NaCl do not contribute substantially to the basic structure of the chromatin bead.

## Discussion

Nucleases have been important probes for studying protein–nucleic acid interactions in chromatin (Murray, 1969; Clark and Felsenfeld, 1971; Mirsky et al., 1972; Hewish and Burgoyne, 1973; Sahasrabuddhe and Van Holde, 1974; Noll, 1974a,b; Weintraub and Van Lente, 1974; Axel et al., 1974; Axel, 1975; Sollner-Webb and Felsenfeld, 1975). Previous studies have revealed regions of differing nuclease sensitivity within chromatin DNA and, along with other biochemical and morphological evidence, have led to the current concept that chromatin is composed of repeating subunits (Kornberg, 1974). The structural and functional characteristics of these chromatin subunits are now being studied in many laboratories.

One point of disagreement concerning the structure of the chromatin subunit is whether all of the DNA of the subunit interacts with the histone core to form a nucleosome (Oudet et al., 1975) or $\nu$ body (Olins and Olins, 1974). The current studies confirm our previous findings that, at low ionic strength, the DNA of the chromatin subunit contains at least two segments having different conformations, and only a portion interacts with the histone core to form the nucleoprotein bead seen in the electron microscope. We also observe that, at physiologic salt concentrations (0.15 M NaCl), chromatin DNA has the same nuclease susceptibility as chromatin DNA in low salt. This observation suggests that the bead and bridge conformation is not an artifact related to conditions of low ionic strength.

We have extended our previous studies by demonstrating that the removal of histone H1 and nonhistone proteins by extraction of chromatin with 0.6 M NaCl primarily affects the properties only of a 40–50 base pair segment of the chromatin subunit DNA, while leaving a 150–160 base pair segment relatively unaffected.

Several observations suggest that the removal of H1 exposes a 40–50 base pair bridge between nucleosomes. First, the initial rate of digestion of H1-depleted chromatin is increased fivefold, suggesting increased availability of the primary nuclease scission sites. Second, multimeric chromatin subunits are virtually never observed during nuclease digestion of H1-depleted chromatin, even after very limited amounts of digestion. This suggests that the nuclease susceptible site(s) between nucleosomes are much more rapidly cleaved in H1-depleted chromatin than in native chromatin. Third, the length of the monomer DNA fragments in H1-depleted chromatin is 150–160 base pairs, the size of the DNA associated with the bead region alone and lacking the 40–50 base pair bridge segment.

These observations are not artifacts related to salt extraction and resuspension of protein-depleted chromatin. When native chromatin is digested and chromatin particles are separated by sucrose gradient sedimentation, the faster sedimenting particles have a DNA length of 180–200 base pairs and contain H1, while the slower sedimenting particles contain only 150–160 base pair DNA and lack H1. Together, these results strongly suggest that H1 affects the properties of the bridge DNA regions between nucleosomes. Nonhistone proteins may also affect the conformation of the bridge region; however, the heterogeneity and paucity of these proteins in HeLa nuclei make it unlikely that they play an important structural role.

Our studies do not reveal how H1 (and/or nonhistone proteins) affects the properties of the 40–50 base pair DNA segment between nucleosomes. One possibility is that H1 binds to the bridge region; removal of H1 would then directly make

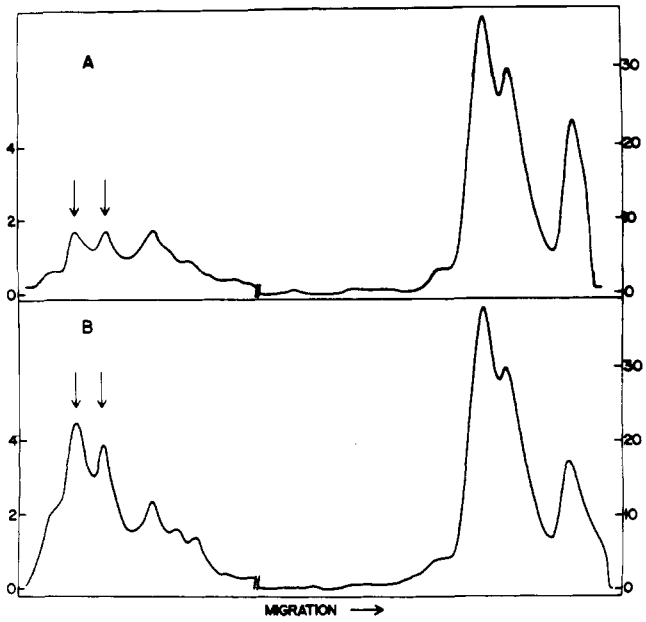


FIGURE 10: Protein was extracted from the fractions indicated in Figure 8 and analyzed on a discontinuous 15% polyacrylamide–sodium dodecyl sulfate gel. The gel was photographed and the negative was scanned. The ordinate is linear with optical density. Note difference in scale between right and left ordinates. The arrows indicate the position of histone H1.

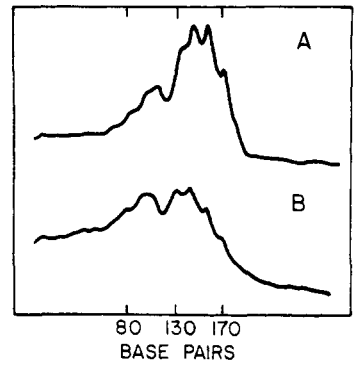


FIGURE 11: DNA fragments in limit digests. Chromatin was extracted with 0.20 mM EDTA plus (A) no salt or (B) 0.60 M NaCl, resuspended in 0.1 mM $CaCl_2$–1 mM Tris (pH 8), and digested with micrococcal nuclease (200 units/ml) at 37 °C for (A) 64 min or (B) 16 min. The DNA was extracted from the resulting limit digest and analyzed on 6% polyacrylamide gels. Densitometer tracings from a negative of the gel photograph are shown. Fragment sizes were estimated by the method of Maniatis et al. (1975).

this DNA segment more nuclease susceptible. On the other hand, H1 may bind adjacent nucleosomes together without any direct interaction with chromatin DNA. Removal of H1 would then lead to a more extended chromatin conformation and a more rapid digestion of the bridge segments. In either case, our results strongly support the concept that the DNA of the chromatin subunit contains at least two segments having different conformations and that the removal of H1 affects primarily the conformation of a 40–50 base pair segment.

H1-depleted chromatin differs in several physiochemical properties from native chromatin: it contains a more clearly resolved low melting DNA component (Hensen and Walker, 1970a); it has a more dispersed appearance in the electron microscope (Mirsky et al., 1968; Oudet et al., 1975). On the other hand, the X-ray diffraction properties (Garrett, 1971), the circular dichroic properties (Hensen and Walker, 1970b), and the hydrodynamic properties (Henson and Walker, 1970a) of chromatin are unaltered by the removal of H1. These observations suggest that H1 does not contribute substantially to the basic structural element of chromatin and is associated with regions of the DNA which have a different conformation than that of the nucleosome.

Our studies confirm the suggestion of Noll et al. (1975) that chromatin prepared using brief nuclease digestion instead of mechanical shearing retains certain properties characteristic of intact nuclei. Such preparations, however, presumably reflect primarily the structural properties of inactive chromatin since only a minor portion of the DNA of a given tissue is transcribed. Studies of fractionated chromatin indicate that H1 is nearly absent from chromatin segments which are active in vitro as templates for DNA-dependent RNA polymerase (Gottesfeld et al., 1974; Simpson, 1974). It seems possible that the removal of H1 from certain bridge regions might expose binding sites for polymerases, regulatory proteins, or other macromolecules involved in the regulation of transcription. On the other hand, the DNA regions containing such recognition sites may be relatively nuclease sensitive, if they are in an extended conformation and relatively protein free. Although chromatin has been fractionated into "transcriptionally active and inactive regions" using DNase II (Gottesfeld et al., 1974, 1975), it remains to be determined whether the functional properties of chromatin, such as the binding of RNA polymerase or regulatory proteins, can be studied using chromatin prepared by a method involving nuclease digestion.

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Added in Proof

Varshavsky et al. (1976) have recently described similar findings.

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# Purification of Myosin Translational Control RNA and Its Interaction with Myosin Messenger RNA†

Stuart M. Heywood* and Doris S. Kennedy

ABSTRACT: Myosin messenger ribonucleoprotein-translational control ribonucleic acid (mRNP-tcRNA) from myosin mRNPs found in embryonic chick muscle has been further purified by Dowex chromatography and, from a number of controls, it is suggested that this small RNA is not an artifact produced through the degradation of RNA during its isolation. This highly purified myosin mRNP-tcRNA is shown to have a molecular weight of 10 000 on formamide-acrylamide gels, and reacts stoichiometrically (on a 1:1 mole ratio) with myosin mRNA. The stoichiometric interaction between myosin mRNA and myosin mRNP-tcRNA is demonstrated by its ability to increase the nuclease resistance of the messenger, as well as inhibit its translation in a cell-free amino acid incorporating system.

The existence of messenger RNA that is not associated with ribosomes in eukaryotic cells is well documented (Brawerman, 1974; Gander et al., 1973; Williamson, 1973). In embryonic muscle both myosin mRNA (Buckingham et al., 1974; Heywood et al., 1975b) and actin mRNA (Bag and Sarkar, 1975) have been shown to exist as ribosomal free mRNP[1] particles in early stages of development. Although it has not been demonstrated that these stored messengers are precursors to polysomal messengers, it is clear that in the case of myosin mRNA, the stored and polysomal forms are identical as determined by hybridization experiments using myosin cDNA (Robbins and Heywood, 1976).

We have suggested that in muscle cells the mechanism by which certain mRNAs are maintained in an inactive form in the cytoplasm is by complexing with a small oligo(U)-containing RNA termed translational control RNA (tcRNA) (Bester et al., 1975). This RNA molecule is characterized by (1) its localization in mRNPs, (2) its oligo(U) region capable of forming hybrids with poly(A), (3) its ability to inhibit the translation of certain mRNAs (those with which it is isolated), and (4) its capability to change the structure of those mRNAs thereby increasing their nuclease resistance. Utilizing these properties of the molecule we have been able to isolate myosin tcRNA from myosin mRNPs and purify it to the extent that it appears as a single band on formamide-acrylamide gel electrophoresis. This highly purified myosin tcRNA was subsequently tested for its ability in one case to hybridize with myosin mRNA and in the other case to interfere with the in

† From The University of Connecticut, Genetics and Cell Biology Section, Storrs, Connecticut 06268. *Received January 19, 1976.* This investigation was supported by Grant HD 03316 from the National Institutes of Health and by Grant CA 14733 from the National Cancer Institute.

[1] Abbreviations used are: mRNP, messenger ribonucleoprotein; poly(A), poly(adenylic acid); DEAE, diethylaminoethyl; EDTA, (ethylenedinitrilo)tetraacetic acid; Hn, heterogeneous nuclear; ATP, GTP, adenosine and guanosine triphosphates.