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Selective Displacement of Histone H1 from Whole HeLa Nuclei: Effect on Chromatin Structure in Situ as Probed by Micrococcal Nuclease†

George M. Lawson‡ and R. David Cole*

ABSTRACT: In order to study the role of histone H1 in chromatin structure under conditions minimizing chromatin denaturation, a technique was developed to displace this histone from whole nuclei. Isolated HeLa nuclei washed at pH 3.0 selectively released histone H1 without detectable displacement of the core histones H2A, H2B, H3, and H4. Nucleosomal structure remained intact in such washed nuclei as judged by the characteristic electrophoretic banding patterns of double-stranded DNA fragments generated by in situ digestion with micrococcal nuclease and pancreatic DNase I, respectively. The patterns were identical with those produced from nuclei maintained at physiological pH. The kinetics of nucleolytic cleavage of the linkage DNA between nucleosome cores in control nuclei containing histone H1 or in nuclei completely or partially depleted of histone H1 were all apparently first order and in each case could be described with a single rate constant. The overall rate of cleavage was increased by a factor of 2.7 ± 0.2 following quantitative displacement of histone H1. A quantitative inverse relationship between the nuclear content of histone H1 and the nucleolytic sensitivity, observed at various degrees of H1 depletion, suggested that the enhanced rate of nucleolysis was in fact dependent upon the displacement of this histone. Calibration of the sizes of resulting DNA fragments indicated that for equal extents of digestion, the fragment sizes were identical regardless of the degree of histone H1 depletion; the ratio of primary endonucleolytic cleavage of linker DNA to subsequent exonucleolytic cleavage was therefore found to be independent of the nuclear content of histone H1. This technique failed to reveal that histone H1 preferentially protected a significant stretch of linkage DNA against exonucleolytic cleavage. It is suggested that the predominant effect on chromatin structure resulting from the displacement of histone H1 under these conditions involves a loosening or dispersal of an otherwise more compact chromatin superstructure in a way that increases the accessibility of the strands of nucleosomes generally rather than directly exposing a specific site on the linkage DNA.

A current model of chromatin structure, derived primarily from histone cross-linking (Kornberg & Thomas, 1974), nuclease digestion studies (Hewish & Burgoyne, 1973; Noll, 1974a), and electron microscopy (Woodcock, 1973; Olins & Olins, 1974; Van Holde et al., 1974a; Oudet et al., 1975), consists of a linear array of repeating protein complexes, linked and circumscribed by a continuous thread of supercoiled duplex DNA. Each protein complex is comprised of two each of histones H2A, H2B, H3, and H4 and chromosomes associated with approximately 200 base pairs of DNA (Kornberg, 1974) to form a subunit of chromatin that has been denoted a nu body or nucleosome. Studies on the precise location of the lysine-rich histone H1 within the strands of nucleosomes have suggested an association of this histone with the DNA that links nucleosomal cores. This suggestion has

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been derived principally from studies in which progressive reduction in the size of the DNA fragment associated with the mononucleosome during the course of nucleolytic digestion was accompanied by a release of histone H1 (Van Holde et al., 1974b; Honda et al., 1975; Bakayev et al., 1975; Varshavsky et al., 1976; Whitlock & Simpson, 1976; Noll & Kornberg, 1977) and from observations on the enhancement in nucleolytic sensitivity of the linkage DNA following displacement of histone H1 from soluble chromatin preparations (Whitlock & Simpson, 1976; Noll & Kornberg, 1977).

Wherever histone H1 is located in chromatin, it is generally thought to participate in the modulation of some level of chromatin condensation, perhaps by functioning as a cross-linker (Littau et al., 1965; Bradbury et al., 1973a; Lewis et al., 1976). Observations correlating the extent of histone H1 phosphorylation with the condensation of interphase chromatin into metaphase chromosomes are consistent with such a role (Lake et al., 1972; Bradbury et al., 1973b; Gurley et al., 1975). Moreover, models have been proposed which implicate histone H1 in the organization of an ordered intrastrand chromatin superstructure by facilitating the stacking of nucleosomes into higher order arrays (Finch & Klug, 1976; Renz et al., 1977; Worcel & Benyajati, 1977).

The comparison of intact chromatin to its histone H1 depleted form is clearly advantageous for identifying the location and function of this histone, but a potential problem with such studies is that the so-called intact chromatin may be denatured in some critical aspect of structure during its isolation. The repeating nucleosome structure is known to be preserved intact in soluble chromatin prepared by limited nuclease digestion of nuclei prior to lysis (Noll et al., 1975), but while this criterion represents a necessary condition of native structure, it might well be insufficient for chromatin preparations to be used in the investigation of levels of chromatin organization of higher order than the nucleosome. The seriousness of the concern is emphasized by the fact that since histone H1 is the least tightly bound of the histones (Ohlenbusch et al., 1967; Jensen & Chalkley, 1968; Murray, 1969; Ilyin et al., 1971) it might be expected that native domains for binding histone H1 would be quite susceptible to disruption by artificial manipulation, particularly shear.

With the hope that the higher order structural features of chromatin in whole nuclei would be better protected against denaturation than in the case of isolated chromatin, we developed a method, based upon a procedure of Mirsky & Silverman (1972), for displacing histone H1 from morphologically intact nuclei. We investigated the kinetics of digestion and the relative sizes of the DNA fragments generated during micrococcal nuclease digestion of histone H1 depleted HeLa nuclei in comparison to nuclei retaining histone H1, either partially or completely. The results indicate that the rate of secondary exonucleolytic cleavage or "triming" following the primary endonucleolytic scission between nucleosomes is dependent upon the presence of histone H1 to the same extent as the primary scission itself. These data are discussed in relation to previous reports on the localization of histone H1 in the chromatin fiber and in terms of a model in which the displacement of histone H1 results in a general increase in accessibility of the nuclease-sensitive linkage DNA following the collapse or unfolding of a higher order chromatin superstructure.

Materials and Methods

Cell Culture. HeLa cells, strain S3, were maintained in suspension culture at cell densities of (2–8) $\times 10^5$/mL in Joklik's modified spinner medium supplemented with 5% calf serum. Cells were generally harvested at densities of (5–6) $\times 10^5$/mL. The stock of cells utilized in these studies was found to be free of mycoplasma as determined by a uridine/uracil uptake assay (Schneider et al., 1974).

Isolation of Nuclei. All steps were performed at 0–4 °C. Pelleted HeLa cells were suspended at a cell density of ca. 10^7/mL in a swelling buffer consisting of 0.1 M hexylene glycol, 1 mM $CaCl_2$, and 0.06 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (pH 6.8) and allowed to swell for 10 min. Cells were disrupted in a loose-fitting Dounce homogenizer, and the crude nuclear pellet was washed 3 additional times in the same buffer by sequential resuspension and centrifugation. Nuclei were then washed once in buffer A made 0.1% in Triton X-100, followed by three washes in buffer A containing no detergent [buffer A: 0.25 M sucrose, 50 mM Tris, 25 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ (pH 7.5)]. Freshly prepared nuclei were used in all experiments. It was found that the stock of cells used in these studies lacked detectable endogenous proteolytic and nucleolytic activities so that proteolytic inhibitors were not required for maintenance of histone integrity and exposure of nuclei to divalent metal ions did not result in predigestion of the DNA.

Extraction of Nuclei in Acidic Buffers. Nuclei were resuspended [(0.6–1.2) $\times 10^7$/mL] in citric acid–sodium phosphate buffers ranging in pH from 2.0 to 3.8 and in total molarity from 50 to 68 mM (Gomori, 1955) and also made 0.25 M in sucrose, 25 mM in KCl, 1 mM in $CaCl_2$, and 1 mM in $MgCl_2$. Nuclei were stirred gently at 4 °C for 15 min unless otherwise specified, collected by centrifugation (300*g*), and extracted a second time under identical conditions. The combined supernatant containing displaced protein was dialyzed against distilled water and lyophilized. The retained histones were solubilized by extracting aliquots of the pelleted nuclei for 4 h at 4 °C in 0.4 N H_2SO_4. The soluble protein was dialyzed against distilled water and lyophilized. Both displaced and retained fractions were analyzed by electrophoresis in 12% discontinuous polyacrylamide gels containing sodium dodecyl sulfate (Laemmli, 1970). Gels were stained with Coomassie Brilliant Blue R-250. Quantitation of histone H1 was performed by scanning the gel lanes at 560 nm in a Guilford recording spectrophotometer equipped with a linear transport device, followed by integration of the scans. Sample loadings were such that the absorbance was approximately linear with respect to quantity of applied sample.

Nuclease Digestion. Following extraction in low-pH buffers, nuclei were washed twice in digestion buffer and resuspended in the same buffer at (1.6–4) $\times 10^7$/mL. Digestion buffers were as follows: for micrococcal nuclease (Worthington), buffer A; for pancreatic DNase I (Sigma), buffer A made 10 mM in $MgCl_2$. Enzyme concentrations are specified in the figure legends. Digestions were carried out at 37 °C and were terminated by addition of EDTA and sodium dodecyl sulfate to 20 mM and 1%, respectively. Following addition of NaCl to 1 M, the aqueous phase was extracted twice with chloroform–isoamyl alcohol (24:1). DNA was precipitated for 16 h at −20 °C by addition of 2 volumes of ethanol. Pellets were washed once in absolute ethanol, dried under vacuum, and dissolved in the appropriate electrophoresis loading solutions.

Gel Electrophoresis of Nuclease Digestion Fragments. DNA fragments from micrococcal nuclease digestions were electrophoresed in 2.5% acrylamide gels according to Loening (1967) as modified to contain 0.5% agarose (Peacock & Dingman, 1968). Electrophoresis was for 3 h at 15 mA in 13 × 14 × 0.1 cm slabs. Fragment sizes were calibrated against a standard plot of migration distance vs. the logarithm of the

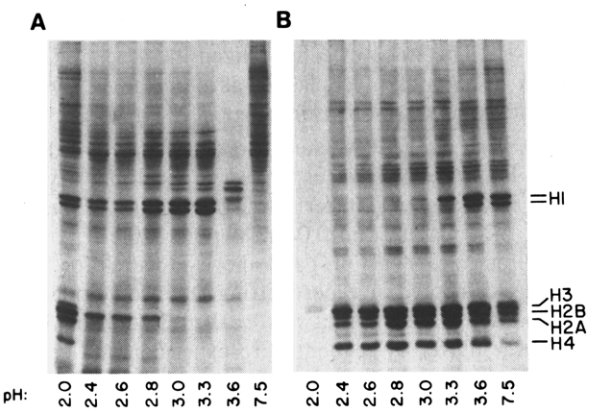

FIGURE 1: Displacement of proteins from intact HeLa nuclei by extraction in acidic buffers. Aliquots of nuclei were extracted twice for 15 min per extraction in citric acid–phosphate buffers of pH as designated in the figure. Nuclei were then gently pelleted (300*g*), and proteins in the supernatant (displaced protein) and pellet (retained protein) fractions were resolved by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate. (A) Displaced protein. (B) Retained protein subsequently solubilized by extraction of residual nuclei in 0.4 N H_2SO_4. Equal loadings (10 μg) of lyophilized material were run on each lane. Migration was from top to bottom.

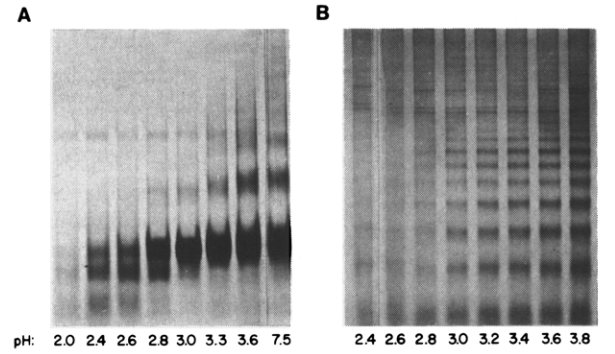

FIGURE 2: Test for the maintenance of nucleosome integrity in HeLa nuclei extracted in acidic buffers. Whole HeLa nuclei were extracted in citric acid–phosphate buffers of pH as designated in the figure, washed twice and resuspended in appropriate digestion buffer (pH 7.5), and digested with nuclease at 37 °C. (A) DNA isolated from nuclei after digestion (1.6×10^7 nuclei/mL) for 15 min with micrococcal nuclease (17 units/mL) and electrophoresed on a 2.5% acrylamide–0.5% agarose nondenaturing gel. (B) DNA isolated from nuclei after digestion (4×10^7 nuclei/mL) for 10 min with pancreatic DNase I (27 units/mL) and electrophoresed on a 12% acrylamide–7 M urea denaturing gel. Micrococcal nuclease digestions were performed on the nuclear preparations described in Figure 1; DNase I digestions were performed on nuclei extracted under identical conditions in a separate experiment. Migration was from top to bottom.

fragment size in base pairs by using fragments generated from digestion of PM2 DNA with *Haemophilus aegyptius* restriction endonuclease III. The sizes of the PM2 restriction fragments taken were those calibrated by Noll (1976). DNA fragments from DNase I digestions were incubated for 5 min at 95 °C in 99% formamide and run in 12% acrylamide–7 M urea denaturing gels as described by Maniatis et al. (1975). Electrophoresis was on slabs for 2 h at 20 mA. Gels were stained overnight in 0.005% Stainsall (Eastman) in 5% formamide and destained in 5% formamide; staining and destaining were performed in the dark. Digestions were quantitated by optical scanning of the gel lanes at 515 nm, followed by integration of the scans. The extent of digestion was taken as the ratio of the monomeric DNA to the total stainable DNA on the gel.

Results

Production of HeLa Nuclei Depleted of Histone H1. During initial attempts to deplete intact HeLa nuclei of histone H1 by extraction in citric acid–NaCl solutions as originally described by Mirsky & Silverman (1972), several difficulties were encountered. First, partial irreversible clumping of the nuclei made them difficult to handle in subsequent steps and introduced a potentially significant variable when comparing the kinetics of nuclease digestion of normal vs. histone H1 depleted nuclei. Second, the extraction conditions also removed some core histones with concomitant disruption of the nucleosome structure as determined by a breakdown in the normally discrete electrophoretic banding pattern given by the DNA fragments derived from limited micrococcal nuclease digestion of the extracted nuclei, an effect presumably resulting from the exposure of additional cleavage sites within the core-associated DNA. The first problem was circumvented by extracting the nuclei in buffers containing sucrose and divalent metal ions. In an attempt to optimize the procedure for selectivity with respect to histone H1 removal, aliquots of nuclei were extracted in a series of citric acid–phosphate buffers of decreasing acidity from pH 7.5 to 2.0. Sodium dodecyl sulfate gel electropherograms of the proteins displaced from the nuclei are shown in Figure 1A along with the complementary patterns of the proteins that remained in the nuclei (Figure 1B). It can be seen that for those extractions carried out in the range from pH 3.6 to 3.0, selective displacement of histone H1 occurred without removal of the other histones; only histone H1 and some nonhistone chromosomal proteins were displaced. At lower pH, however, histone H2B was partially lost (between pH 2.8 and 2.4), and finally at pH 2.0 the majority of the core histones were released. Throughout the entire range of pH, the nuclei remained morphologically intact as judged by phase-contrast microscopy and by recovery of DNA (precipitable in 7% perchloric acid) from low-speed (2 min at 300*g*) pellets of extracted nuclei.

The effect of acidic extraction conditions on the maintenance of nucleosome integrity was investigated by digesting aliquots of the extracted nuclei with micrococcal nuclease, followed by electrophoretic analysis of the resulting DNA digestion fragments. From nuclei extracted at pH 3.0 and above, a single broad band characteristic of the mononucleosome was obtained as shown in Figure 2A. Digestion of nuclei extracted at any pH below pH 2.8–3.0 resulted in a partial breakdown of the monomeric fragment and simultaneous generation of more rapidly migrating components presumably due to the exposure of additional nuclease-susceptible sites. To further test the extent of nucleosome disruption over the pH range in question, nuclei were extracted at different pH values and digested with pancreatic DNase I. Since this enzyme has been shown to cleave within the nucleosome by introducing staggered single-strand nicks at integral multiples of 10 nucleotides (Noll, 1974b), the generation of such a discrete intranucleosomal cleavage pattern provides a test for the native state of the nucleosome. There appear to be some qualifications to this test since Liu & Wang (1978) report that a pattern of multiples of 10 nucleotides is obtained by DNase I digestion of DNA lying on any surface. Nevertheless, as seen in Figure 2B, a discrete cleavage pattern that was well maintained with nuclei extracted at pH 3.0 or above showed deterioration where the extraction had been carried out below pH 3.0. Evidently, these patterns are sensitive to some form of chromatin denaturation. Moreover, it is especially important to note that the pattern of preferences among DNase I cleavage sites was not noticeably affected by removal of H1 histone (Figure 2B). Taken together these observations support the contention that the structure at the level of the nucleosome strand remains

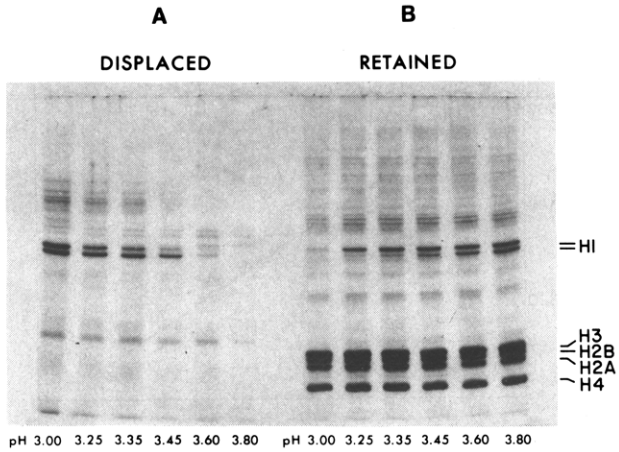

FIGURE 3: Titration of histone H1 from whole HeLa nuclei by extraction in acidic buffers. Aliquots of nuclei were extracted in citric acid–phosphate buffers of pH as designated in the figure. Nuclei were then gently pelleted (300*g*), and proteins in the supernatant (displaced protein) and pellet (retained protein) fractions were resolved by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate. (A) Displaced protein. (B) Retained protein subsequently solubilized by extraction of residual nuclei in 0.4 N H_2SO_4. Equal loadings (10 μg) of lyophilized material were run on each lane.

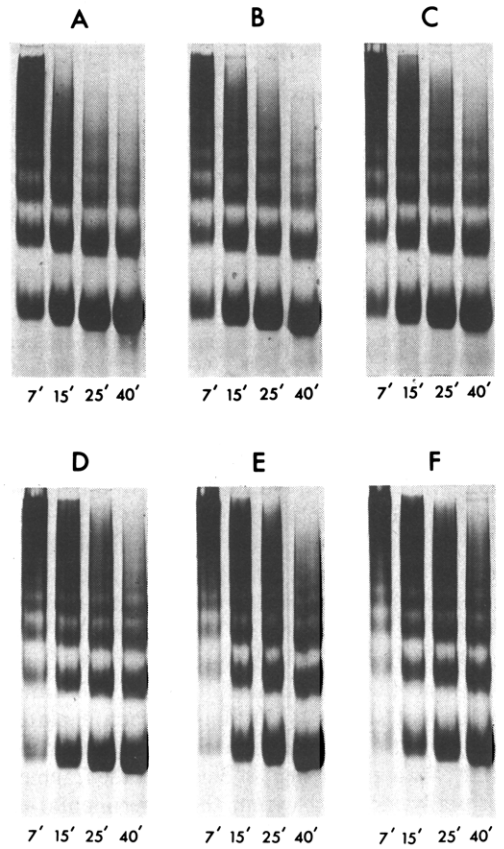

FIGURE 4: Micrococcal nuclease digestion of HeLa nuclei depleted of histone H1 to various extents. Nuclei extracted in citric acid–phosphate buffers at pH (A) 3.0, (B) 3.25, (C) 3.35, (D) 3.45, (E) 3.6, and (F) 3.8 were washed twice and resuspended (2.5×10^7 nuclei/mL) in buffer A (pH 7.5) and then digested with micrococcal nuclease (4.3 units/mL) at 37 °C for increasing times. DNA digestion fragments were resolved by polyacrylamide–agarose gel electrophoresis. Digestion times are indicated in the figure. The histone complement retained by nuclei after extraction in the acidic buffers is presented in Figure 3.

intact with HeLa nuclei exposed to buffers as acidic as pH 3.0.

In order to determine the minimum time required for complete displacement of histone H1, nuclei were extracted twice at pH 3.0 for 5, 15, or 30 min per extraction. Since maximal displacement was achieved after two 15-min extractions, these conditions were used for all subsequent studies.

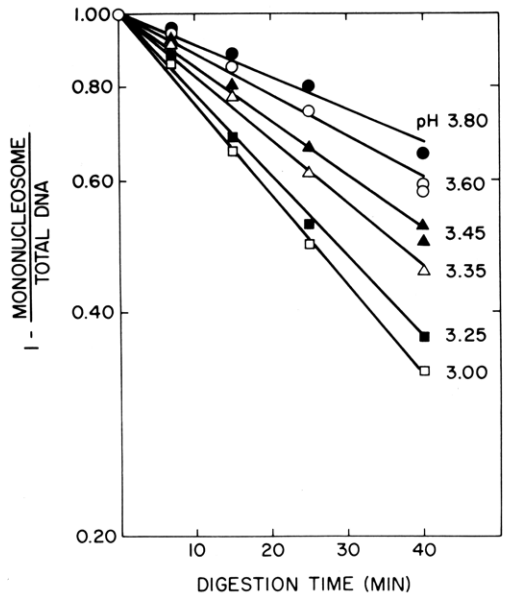

FIGURE 5: Effect of histone H1 displacement from HeLa nuclei on the kinetics of nuclease digestion. Optical scans of the DNA gels presented in Figure 4 were integrated, and the extent of digestion at each time point was taken as the fraction of total stainable DNA on a lane existing as monomeric DNA. Data were plotted as shown to demonstrate apparent first-order kinetics of digestion. The pH of the nuclear extraction buffer is designated in the figure.

Kinetics of Nuclease Digestion of Histone H1 Depleted Nuclei. It was of interest to determine what effect the depletion of histone H1 would have on the overall rate of digestion of intact nuclei by micrococcal nuclease and also on the relative sizes of the DNA digestion fragments. Aliquots of nuclei were extracted over the range pH 3.0–3.8 and digested with micrococcal nuclease for various times. Sodium dodecyl sulfate gels of the displaced protein as well as the protein that was not extracted from the nuclei are presented in Figure 3. It is evident that an increasing displacement of histone H1 from the nucleus was observed as the buffer pH was decreased over this attenuated range, producing nuclei depleted of histone H1 to various degrees. The companion DNA gels are presented in Figure 4. Scans were obtained directly from the gels and integrated; the extent of digestion at each time point was taken as the ratio of the monomeric DNA to the total stainable DNA on the lane. The data were tested for first-order kinetics of digestion by plotting the logarithm of the undigested DNA [1.0 − (monomer/total DNA)] against the time of digestion as shown in Figure 5. In agreement with Seale (1976), it is seen that nuclei containing a full complement of histones (i.e., pH 3.8 extracted nuclei) yielded a straight line, indicative of apparent first-order digestion kinetics. For nuclei extracted at a series of lower pH values, a progressive increase in the rate of digestion was observed, and apparent first-order kinetics were observed over the entire range of histone H1 depletion. It should be emphasized that the nuclei were thoroughly washed with digestion buffer (pH 7.5) prior to digestion so that the observed difference in kinetics cannot be attributed to differential nuclease activity due to variations in pH during digestion.

The protein gels displayed in Figure 3 were scanned and integrated in order to measure the fraction of histone H1 retained. The percentage of histone H1 retained in each preparation was calculated relative to histone H4 which was

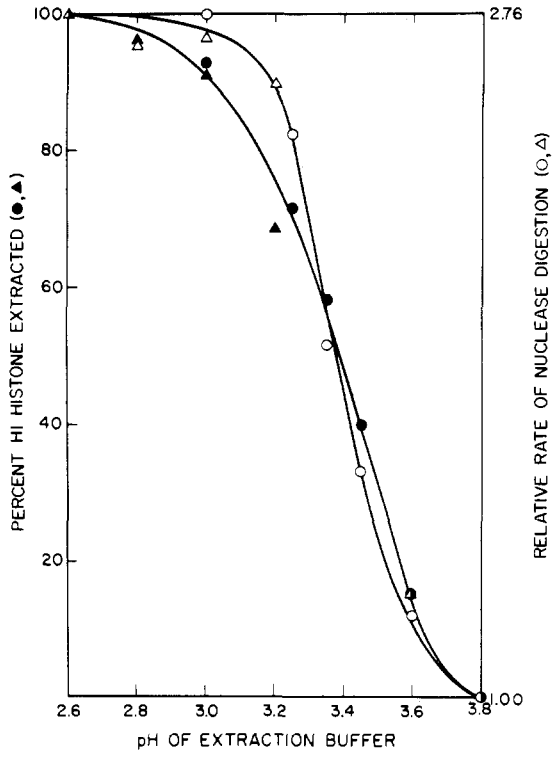

FIGURE 6: Relationship between the percentage of histone H1 displaced from HeLa nuclei and the apparent first-order rate constant of nuclease digestion. Apparent first-order rate constants were calculated from the kinetic data presented in Figure 5. The scale for the relative rate of nuclease digestion (○) was normalized by assigning a value of unity to the rate constant calculated from nuclei extracted at pH 3.8 and adjusting the other rate constants proportionally. The percentage of histone H1 displaced (●) was obtained from optical scans of the protein gel displayed in Figure 3. (△ and ▲) Relative rate of nuclease digestion and percentage of histone H1 displaced, respectively, from a separate experiment.

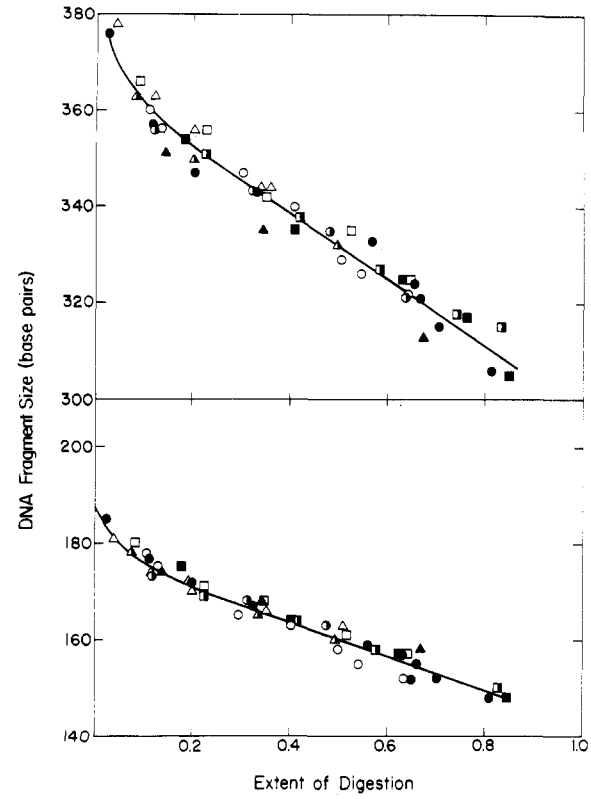

FIGURE 7: Size calibration of DNA fragments produced by nuclease digestion of HeLa nuclei depleted of histone H1 to various extents. The mean size, in base pairs, of monomeric (lower panel) and dimeric (upper panel) DNA fragments generated by micrococcal nuclease digestion of nuclei extracted in citric acid–phosphate buffers of different pH plotted as a function of the extent of digestion, which was taken as the fraction of the total DNA on a lane existing as monomeric DNA in both cases. Calibration was against standard curves constructed from the mobilities of PM2 DNA restriction fragments electrophoresed on the same gel. The pHs of the nuclear extraction buffers were as follows: (●) pH 2.8; (▲ and ■) pH 3.0 (two experiments); (□) pH 3.2; (◑) pH 3.25; (△) pH 3.45; (□) pH 3.6; (○ and △) pH 3.8 (two experiments).

well resolved in this electrophoretic system and was completely retained by nuclei over the pH range under study. The correlation between the histone H1 displacement and the increase in the rate constant of DNA digestion is emphasized in Figure 6 by plotting each of these parameters as a function of the pH of nuclear extraction. Also included are data points derived from a separate experiment in which nuclei were extracted in the pH range 2.6–3.6. The scale for the rate constant was normalized by assigning a value of unity to the apparent first-order rate constant of digestion calculated from nuclei extracted at pH 3.8 and adjusting the other rate constants proportionally. The rate of digestion was identical for nuclei extracted at pH 3.8 and 7.5 (data not shown). Combining all the data from several experiments, we observed that removal of histone H1 increased the first-order rate constant of digestion by a factor of 2.7 ± 0.2 (pH 3.0 vs. pH 3.8 extracted nuclei).

Size Calibration of DNA Digestion Fragments. In order to ascertain possible effects of histone H1 depletion on the sizes of the DNA fragments generated during the course of in situ digestion by micrococcal nuclease, the DNA preparations presented in Figure 4 as well as similar preparations from two additional experiments were electrophoresed in parallel with PM2 DNA fragments which had been produced by digestion with *H. aegyptius* restriction endonuclease III. As seen from Figure 4, for nuclei extracted at a given pH, the momomeric fragments decrease in size as a function of digestion time. This observation is consistent with numerous other reports (Noll, 1974a; Whitlock & Simpson, 1976; Noll & Kornberg, 1977; Lohr et al., 1977) and has been attributed to an exonucleolytic or "nibbling in" activity subsequent to the primary endonucleolytic cleavage between nucleosomes. In order to control for this effect when comparing fragments generated from histone H1 depleted and histone H1 containing nuclei, the fragment size (in base pairs) was plotted as a function of the overall extent of digestion (monomer DNA/total DNA). The data for monomeric and dimeric DNA digestion fragments are presented in Figure 7. In agreement with Lohr et al. (1977), we find that in the case of HeLa, the dimer (and trimer—data not shown) sizes fail to reach a plateau but continue to decrease over the entire range of digestion studied. Significantly, for a given extent of digestion, the fragment sizes were essentially identical within the experimental error of calibration regardless of whether they were generated from nuclei containing the normal complement of histone H1 or nuclei either completely or partially depleted of histone H1. The data show not only that the rates of nucleolysis were augmented in proportion to the amount of histone H1 displaced but also that the rates of endonucleolytic and exonucleolytic digestion (Noll & Kornberg, 1977) were equally affected at any level of histone H1 removal.

Discussion

We have described a method for the selective extraction of histone H1 from intact HeLa nuclei by mildly acidic washes. Acid extraction was used previously for the depletion of histone H1 from whole nuclei, but no biochemical criteria were applied

to assess denaturation of the residual chromatin (Littau et al., 1965; Mirsky & Silverman, 1972; Brasch et al., 1972; Tanaka & Oda, 1976). Under the conditions described here, the nucleosome appears to remain intact as judged by the discrete electrophoretic DNA banding patterns generated by in situ digestion with micrococcal nuclease and DNase I. Further evidence that nucleosomes can withstand such treatment comes from experiments in which nuclei exposed to a mildly acidic milieu exhibited an X-ray diffraction pattern characteristic of native chromatin (Olins & Olins, 1972). Also, brief extractions of chromatin in acidic solutions at low temperature did not result in depurination of the DNA (Murray, 1969). It thus appears that despite the low pH required for histone H1 displacement, the chromatin within the nucleus remains native in many respects. Moreover, the fact that the H1 depleted nuclei were essentially the same size as normal nuclei (by phase-contrast and electron microscopy) indicated that the chromatin did not undergo profound volume changes during histone H1 depletion and that the shear force exerted on the chromatin during isolation, extraction, and digestion of the nuclei was minimal.

The rate of cleavage of the linkage DNA increased approximately 2.7-fold upon complete removal of histone H1. Since some nonhistone chromosomal proteins were displaced along with histone H1, their removal might also have contributed to the augmentation of nucleolytic sensitivity. However, the proportionality between the histone H1 displaced and the susceptibility to nucleolysis suggests that the major effect can probably be attributed to the release of this histone. Even here, the suggestion must be qualified since a few of the nonhistones were released from HeLa nuclei in proportion to the H1 histone. In two other cell lines, however, where the buffering range over which histone H1 was completely titrated from the nucleus was shifted to more acidic values (pH 2.7–2.8 required for 90% displacement), the increase in nucleolytic sensitivity was similarly shifted and still coincided with the displacement of histone H1 (data not shown).

The increase in rate of nucleolysis upon histone H1 displacement documented here is 2–3 times less than in previous reports where soluble chromatin was depleted of H1 histone by 0.6 M NaCl or excess tRNA (Whitlock & Simpson, 1976; Noll & Kornberg, 1977). Explanations for this discrepancy include the state of the chromatin (i.e., soluble or intranuclear) or the method used to strip the H1 histone. With regard to the latter, we have observed the onset of nuclear lysis and gelation of the chromatin upon suspension of HeLa nuclei in 0.3 M NaCl; among other possibilities, this might suggest that elevated ionic strengths affect chromatin structure in ways beyond those related to histone H1 displacement. Rearrangement of core histones was in fact reported to occur in 0.6 M NaCl (Varshavsky & Ilyin, 1974). It is not clear how to compare the electrostatic effectiveness of low concentrations of tRNA to that of high (0.6 M) concentrations of monovalent salts, but cooperativity of its many negative charges might well render tRNA less gentle than is sometimes supposed. In any case, although transport of core histones onto exogenous nucleic acid is minimal under the conditions normally employed to displace lysine-rich histone with tRNA (Ilyin et al., 1971), a detectable broadening in the electrophoretic bands yielded by DNA digestion fragments generated from such chromatin has been noted (Noll & Kornberg, 1977); this effect may be related to the reduced resolution in the DNA fragment pattern reported for chromatin preparations subjected to mechanical shear (Noll et al., 1975). Perhaps removal of histone H1 produces a lability in the residual chromatin fiber so that subsequent manipulation, perhaps aggravated by salt, might induce additional structural perturbations superimposed upon those most directly related to histone H1 depletion. Whether the reason is that dilute acid (mM) is more gentle than concentrated salt or tRNA or that the nuclear structure protects the physical conformation of chromatin against the hostile environment of an aqueous solution, it is a fact that when histone H1 is extracted at pH 3 from nuclei, the electrophoretic patterns of monomeric and oligomeric DNA digestion fragments, including widths of the bands as well as their spacings, are not detectably different from the corresponding fragments given by untreated nuclei. This suggests that the relative orientation of nucleosomes along the DNA remains unaltered in nuclei depleted of lysine-rich histone as described here.

Size calibration of DNA digestion fragments as a function of the extent of primary cleavage indicated that, within limits of detection, the ratio of endonucleolytic to subsequent exonucleolytic cleavage rates was independent of nuclear histone H1 content. Significantly, following the primary cleavage event, the rate of exonucleolytic cleavage down to 140 base-pair core DNA in nuclei depleted of histone H1 was comparable to that in control nuclei retaining a full complement of H1 histone. These data therefore failed to reveal a direct protection against exonucleolytic attack conferred by the H1 histone to an extensive segment of the linkage DNA. This does not rule out the binding of H1 histone to a particular portion of linkage DNA; it simply indicates that the protection afforded by such binding is trivial when compared to the protection afforded by higher order folding of the chromatin.

The lower rate of internucleosomal scission observed for control as compared to histone H1 depleted nuclei is not necessarily dependent upon direct complexing of the histone with the primary cleavage site. A reasonable alternative is that the displacement of histone H1 is accompanied by a general loosening of the chromatin so that the primary cleavage sites become generally more accessible to anything in the aqueous environment. Electron microscopy of interphase chromatin has revealed an attenuation in fiber diameter and the simultaneous appearance of "beaded-string" structures following the removal of histone H1 (Oudet et al., 1975; Finch & Klug, 1976; Renz et al., 1977; & Benjayati, 1977; Thoma & Keller, 1977; Vengerov & Popenko, 1977). Sedimentation analysis also suggested an extension of oligomeric chromatin fragments stripped of histone H1 (Noll & Kornberg, 1977; Renz et al., 1977; Harrington, 1977). Those results, like the ones presented here, imply that histone H1 may play an active role in promoting a localized packing of nucleosomes into more compact structural arrays. It is therefore concluded that the predominant effect on chromatin structure resulting from the displacement of histone H1 from whole nuclei appears to involve a loosening or collapse of an otherwise more compact chromatin superstructure in a way that increases the accessibility of the DNA to nuclease generally rather than directly by exposure of an H1 binding site to nucleolysis.

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