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Structure of the Chromatosome, a Chromatin Particle Containing 160 Base Pairs of DNA and All the Histones†

Robert T. Simpson

ABSTRACT: I have prepared chromatin particles from chicken erythrocytes which contain a 160 base pair length of DNA, an octamer of the four smaller histones, and a molecule of lysine rich histone, H1 or H5, and have compared some physicochemical properties of these particles with those of core particles, which lack H1 or H5 and contain 20 base pairs less of DNA. The former particles, called chromatosomes for convenience, are of particular interest in that they contain all the major components necessary for organization of the structure of long stretches of chromatin. Assuming that the structure of the 140 base pairs of DNA common to the core particle and the chromatosome is similar in these two particles, physicochemical data suggest that (1) the additional DNA in the chromatosome is folded around the histone core, (2) the additional DNA is not in the same conformation as the 140 base pairs of DNA in the core particle, and (3) both the 20 additional base pairs of DNA in the chromatosome and some DNA segments of the core particle itself are markedly stabilized to thermal denaturation, presumably by interaction with the lysine rich histone. Using 5′-end labeling, I have mapped the relative susceptibilities of DNase I cleavage sites in the chromatosomes. Sites at 20, 50, 60, and 100 bases from the ends are highly susceptible; the sites 30, 40, and 70-90 bases from the ends are cleaved with lower frequency. These data suggest that the chromatosome likely contains two full turns of DNA around the histone nucleus of the core particle, with a molecule of lysine rich histone bound outside the DNA-inner histone complex.

When chromatin is degraded by micrococcal nuclease, there are several levels of structural organization from which nucleoprotein particles can be isolated and studied with regards to their composition and structure; all of these are metastable intermediates in the course of a hydrolytic reaction which leads from the intact, high molecular weight DNA of the cell nucleus to the limit digest, at which point about half of the DNA has been degraded to acid solubility. The major pauses in the degradative process occur at: (1) a 40S particle consisting of about eight nucleosomes in a compact structure (Hozier et al., 1977); (2) the nucleosome, a particle containing the biochemical repeat length of DNA in the particular tissue, usually 185-205 base pairs (bp) of DNA (Noll, 1974; Axel, 1975; Sollner-Webb & Felsenfeld, 1975; Shaw et al., 1976; Noll & Kornberg, 1977); (3) a particle, which, for convenience, I will call a chromatosome, containing about 160 bp of DNA, one molecule of H1 (or H5), and an octamer of the four smaller histones (Varshavsky et al., 1976; Whitlock & Simpson, 1976a; Bakayev et al., 1977; Todd & Garrard, 1977; Noll & Kornberg, 1977); and (4) the core particle, consisting of 140 bp of DNA and the inner histone octamer, but lacking any lysine rich histone. The last of these species has been subjected to intensive physicochemical characterization, leading to a fairly detailed understanding of its structure (for reviews, see Kornberg, 1977; Felsenfeld, 1978). In progressing from our current understanding of the core particle towards an eventual understanding of the overall organization of chromatin, it seems that the structure of the chromatosome might be of particular interest, since this particle contains all the major components necessary for the structural organization of long stretches of chromatin, all the histones and DNA. While the structure and role of the apparently variable length of linker DNA which connects such particles remain uncertain, it should be noted that certain species have biochemical repeat lengths of near 160 bp of DNA (Morris, 1976; Noll, 1976; Lohr et al.,

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1977), and that chromatosomes have been identified in nuclease digests from a number of tissues (references cited above), suggesting that the chromatosome may be the basic repeating structural unit in the chromosome.

A major compositional difference between the core particle and the chromatosome is the presence of one molecule/particle of lysine rich histone (H1 or H5) in the latter. The role of these histones in the structure of chromatin has not been elucidated, but it seems to be related to the degree of condensation of the nucleoprotein; removal of H1 leads to decondensation of chromatin as viewed in the electron microscope (Langmore & Wooley, 1975; Oudet et al., 1975; Finch & Klug, 1976; Christiansen & Griffith, 1977; Renz et al., 1977; Thoma & Koller, 1977) and as detected by hydrodynamic measurements (Noll & Kornberg, 1977). These findings would suggest a major role for the lysine rich histones in interparticle interactions. The possible role of H1 or H5 in the structure of the nucleosome per se has not apparently received attention, even though the observation that particles containing 160 bp of DNA may also contain H1 (Bakayev et al., 1977; Todd & Garrard, 1977; Noll & Kornberg, 1977) would suggest that this histone might interact with either the subunit DNA, the histone nucleus of the core particle, or both.

I have prepared from chicken erythrocytes a chromatosome fraction in about 90% purity. These particles contain 160 bp of DNA, an octamer of the four smaller histones, and a near-stoichiometric amount of lysine rich histone (H5 + H1). I have studied the physical properties of and the organization of DNA in these particles. The results suggest that the chromatosome contains 10 bp of DNA on each end of the core particle segment and that the lysine rich histone interacts not only with this additional DNA, but also with the DNA near the ends of the core particle segment and possibly with the octameric histone nucleus of the chromatin subunit.

Experimental Section

Preparation of Chromatosomes and Core Particles. Nuclei were prepared from chicken erythrocytes (Pel-Freez Biologicals) as previously described (Whitlock & Simpson, 1976b). Nuclei at A_{260} = 100 in 0.25 M sucrose, 10 mM Tris-Cl, pH 8.0, 1 mM $CaCl_2$ were digested with micrococcal nuclease (Worthington Biochemicals Corp.) at a concentration of 200 U/mL at 37 °C for 30 min. Digestion was terminated by the addition of EDTA to a final concentration of 20 mM, cooling to 0 °C and sedimentation of the nuclei at 3000*g* for 10 min. The nuclear pellet was resuspended in 0.25 mM EDTA, pH 7.0, using a volume half that of the original suspension, and dialyzed for 16 h against the same buffer. Debris was removed by centrifugation at 3000*g* for 15 min and the supernatant adjusted to A_{260} = 100. Aliquots were fractionated by sedimentation on isokinetic sucrose gradients (C_m = 5%, particle density 1.51 g/cm^3 at 4 °C (McCarty et al., 1974) containing 25 mM Tris-Cl, pH 8.0, 1 mM EDTA at 25 000 rpm for 20–22 h at 4 °C in a Beckman SW27 rotor. Gradients were emptied by pumping from the bottom of the tubes through a flow cell in a Beckman DB-G spectrophotometer. The monomer peak (nominally 11S) was collected, dialyzed against 0.1 mM phenylmethanesulfonyl fluoride, 1 mM EDTA, pH 8.0, and concentrated to 2–3 mg of DNA/mL by dialysis against dry Sephadex G-200 (Pharmacia Corp.). To fractionate the preparation, I used the observation that core particles are soluble in solutions of ionic strength 0.1, while particles containing H1 and H5 are not (Olins et al., 1976; Whitlock & Simpson, 1976b). KCl (1 M) was added to the monomer fraction to a final concentration of 0.1 M and, after incubation at 0 °C for 30 min, chromatosomes were pelleted by centrifugation at 3000*g* for 10 min. The supernatant, containing core particles, was dialyzed to 0.25 mM EDTA, pH 7.0, concentrated if necessary and stored frozen. The pellet was redissolved in 0.25 mM EDTA, pH 7.0, dialyzed against the same solvent, and frozen. The core particles contain as a major species DNA of length 138 base pairs, 1.2 times the DNA mass of the four smaller histones, in equal molar proportions, and no H1 or H5. The fraction precipitated by 0.1 M KCl contains 90% DNA of length 160 bp and 10% DNA of length 120 bp, 1.22 times the DNA mass of histones, and nearly stoichiometric amounts of H1 + H5 histone (see below).

When necessary, lysine rich histones were removed from chromatosomes by adjusting the particles to a NaCl concentration of 0.45 M and sedimentation through isokinetic sucrose gradients containing 0.4 M NaCl. Generally, this procedure was carried out with a SW41 rotor for 16–18 h at 37 000 rpm and 4 °C. Samples were collected, concentrated, and stored as detailed above.

Methods for Analysis of Proteins and DNA. Histones were analyzed as previously described (LeStourgeon & Rusch, 1973; Whitlock & Simpson, 1976b). DNA was purified by phenol extraction and analyzed as previously described, using 5% polyacrylamide gels for resolution of native, double-stranded DNA and 12% polyacrylamide gels containing 7 M urea for resolution of denatured, single-stranded DNA fragments (Maniatis et al., 1975; Simpson & Whitlock, 1976). Size markers for native DNA gels were an *Hae*III digest of ΦX174 RF DNA (Bethesda Research Labs.).

For determination of rates of DNase I digestion and susceptibilities of cleavage sites in various chromatin particles, samples were labeled at the 5′ end of the DNA using [γ-^{32}P]ATP (New England Nuclear Corp.) and polynucleotide kinase (Miles Biochemicals) as previously described (Simpson & Whitlock, 1976). Scans of autoradiograms of the labeled DNA demonstrated that (1) DNA was not degraded during the modification and (2) the amount of ^{32}P incorporated into the 160- and 120-bp fragments was proportional to their representation in the total DNA present. Forty to seventy percent of the 5′ ends were labeled with ^{32}P. After digestion with DNase I (Worthington Biochemical Corp.) in 10 mM Tris-Cl, pH 8.0, 10 mM $MgCl_2$, at 37 °C, aliquots were precipitated with 10% perchloric acid, and centrifuged, and supernatants counted to determine the fraction of the label made acid soluble. Other aliquots were subjected to deproteinization and electrophoresis, staining and autoradiography as previously described (Simpson & Whitlock, 1976).

DNA concentrations were estimated by assuming the absorbance at 260 nm of a 1 mg/mL solution of the nucleic acid in nuclei or chromatin particles is 20. Protein contents were measured by the method of Lowry et al. (1951) with calf thymus histones as a standard.

Physical Methods. Thermal denaturation of various chromatin samples was followed in a Beckman ACTA III spectrophotometer using a platinum resistance thermometer in a dummy cell for temperature measurement and a NesLab temperature programmer to provide a linear temperature increase. The platinum thermometer was calibrated against a mercury thermometer. The thermal transition curves were smoothed, digitized using a Numonics Corp. graphics calculator and differentiated using a linear least-squares fitting program for five data points around each experimental measurement. Circular dichroism measurements were performed as previously described (Simpson & Sober, 1970).

Sedimentation analyses were performed in a Beckman Model E analytical ultracentrifuge equipped with ultraviolet scanner and multiplexer accessories. Observed sedimentation

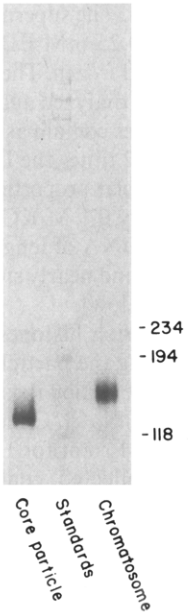

FIGURE 1: DNA lengths in core particle and chromatosome fractions. DNA isolated from the 11S sucrose gradient fraction soluble in 0.1 M KCl (left track) or insoluble in 0.1 M KCl (right tract) was electrophoresed on a 5% polyacrylamide gel together with a *Hae*III digest of θX197 RF DNA (center track) as size standards. The sizes of the three smallest standards visible are 118, 194, and 234 base pairs, as indicated.

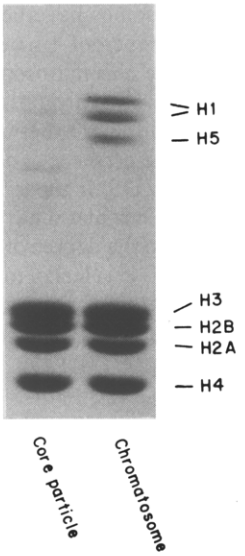

FIGURE 2: Histones in core particle and chromatosome fractions. Histones isolated from the 11S sucrose gradient fraction soluble in 0.1 M KCl (left track) or insoluble in 0.1 M KCl (right track) were electrophoresed on discontinuous 18% polyacrylamide gels containing 0.1% sodium dodecyl sulfate. Positions of migration of the histones are indicated. Quantitative densitometry of the stained gel showed the stain intensity of H5 + H1 to be 22% of the stain intensity of H2A + H2B + H3 + H4.

velocities, analyzed as least-square regression lines, had standard deviations of the slopes of less than 1% of the regression coefficient. The data were corrected to a solvent with the density and viscosity of water at 20 °C.

Results

Isolation and Composition of Chromatosomes. We and others have noted that the 11S fraction derived from micrococcal nuclease digestion of chromatin is heterogeneous when sedimented in sucrose gradients or when examined on nucleoprotein gels (Olins et al., 1976; Varshavsky et al., 1976; Whitlock & Simpson, 1976a; Bakayev et al., 1977; Todd & Garrard, 1977; Noll & Kornberg, 1977). When prepared under the conditions described in the Experimental Section, the 11S peak in a micrococcal nuclease digest of chicken erythrocyte nuclei contains a mixture of nucleoprotein fragments, containing different lengths of DNA. Nearly equal amounts of 140- and 160-bp length nucleic acid are present; additionally a small amount of DNA about 120 bp in length is found. This observation has been made at several stages of digestion (<10–35% acid-soluble A_{260}). The four smaller histones are present in approximately equimolar proportions, but, in addition, lesser amounts of H1 and H5 are also contained in such preparations. Fractionation of this 11S fraction by precipitation with 0.1 M salt leads to resolution of two populations of particles. The fraction soluble in 0.1 M KCl contains only the four smaller histones and a distribution of DNA centered near 140 bp in length with some tailing to longer sizes (Figures 1 and 2). In contrast, the fraction precipitated by 0.1 M KCl has a major DNA band of length 160 bp, nearly as homogeneous as core particle DNA, a minor DNA band at 120 bp (Figure 1), equimolar amounts of the inner histones, and an amount of H1 + H5 nearly equal on a mass basis (as judged by stained band intensity), to the amount of any of the four smaller histones (Figure 2). Some selection in the monomer fraction is evidenced by the observation that the chromatosome fraction shown contains more H1 than H5; the reverse is true for erythrocyte nuclei. This is due to an apparent difference in the rates of digestion of chromatin segments containing H1 vs. H5. At early stages in the digestion, the monomer peak contains predominantly H5; later, as shown here, H1 is the major lysine-rich histone in the monomer fraction. The content of the 120-bp fragment in several preparations was about 10% of the total material precipitable by 0.1 M KCl.

Analysis of the protein contents of the fractionated particles confirms the impression from the stained gels that the major portion of the 0.1 M KCl insoluble fraction is a 160-bp length of DNA associated with two each of the four smaller histones and one molecule of H1 or H5. The protein content of the core particles is 1.20 ± 0.03 g/g of DNA, as expected from the mass of DNA (92 000 daltons) and histones (110 000 daltons) known to be present in these well-defined particles. The protein content of the chromatosome fraction is 1.22 ± 0.03 g/g of DNA, again consistent with the presence of an octamer of the four smaller histones and one molecule of H1 or H5 (132 000 daltons) and a 160-bp length of DNA (105 000 daltons). Sedimentation of these particles in sucrose gradients containing 0.4 M NaCl decreases their protein content to 1.02 ± 0.02 g/g of DNA, indicating the complete removal of the lysine rich histones, a finding confirmed by gel electrophoretic analyses (data not shown).

Physical Properties of Chromatin Particles. I have compared several physical properties of these three classes of particles: (1) core particle; (2) chromatosome; and (3) chromatosome (−H1, −H5), the salt washed chromatosomes lacking the lysine rich histones. Sedimentation velocity analysis demonstrates that all three particles are compact and nearly globular in overall conformation (Table I). All particles sedimented as symmetrical boundaries at an ionic strength of 15 mM, with the exception of a minor (10%), more rapidly sedimenting species in the chromatosome preparation. The sedimentation coefficients for the three particles were 10.7, 11.7, and 11.0, respectively. The value for core particles is in good agreement with determinations from our and other laboratories; this sedimentation coefficient reflects the generally globular overall structure of the core particle (Olins et al.,

TABLE I: Physical Properties of Chromatin Particles.[a]

particle	$s_{20,w}$ (S)	$(\theta)_{282}$ (deg cm^2/dmol of PO_4)
core particle	10.7 ± 0.05	1300
chromatosome	11.7 ± 0.06	1800
chromatosome (−H1, −H5)	11.0 ± 0.07	1900

[a] Physical measurements were made in 10 mM KCl, 5 mM Tris-Cl, pH 8.0, 0.1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride. Error limits on sedimentation coefficients are the standard deviations of the calculated linear least-squares slope fitting the data. Noise levels for the circular dichroism measurements are approximately ±100 deg cm^2/dmol of phosphate.

1976). The sedimentation coefficients for chromatosomes and chromatosomes (−H1, −H5) indicate that the additional DNA and histone in these particles are also folded into a relatively compact conformation.

Circular dichroism measurements provide a different means of assessment of the conformation of DNA in a nucleoprotein. Compared with the circular dichroism spectrum of protein free DNA, with an ellipticity at the positive maximum near 280 nm of 9000 deg cm^2/dmol of phosphate, the ellipticity of chicken erythrocyte core particle DNA is markedly reduced, to 1300 deg cm^2/dmol of phosphate (Table I). While the physical basis for this altered spectrum is not currently known, it has been shown to be highly characteristic of the conformation in which DNA exists in the core particle (Whitlock & Simpson, 1976b; Stein et al., 1977; Weischet et al., 1978). Both the chromatosome and the chromatosome (−H1, −H5) have higher ellipticities in this spectral region, 1800 and 1900 deg cm^2/dmol of phosphate, respectively (Table I). Assuming that the conformation of the 140 bp of DNA common to core particle and chromatosome is the same in both particles, the increase in ellipticity suggests that the additional 20 bp of DNA in the chromatosome (vs. the core particle) are not in the same conformation as core particle DNA. Further, the conformation of these additional DNA regions is likely not that of isolated B form DNA in solution, since the ellipticity of the chromatosome is less than that predicted for a linear combination of 140 bp of DNA in a core particle conformation and 20 bp of DNA in B form structure (the predicted value is 2250 deg cm^2/dmol of phosphate). Finally, whatever the conformation of the additional 20 bp of DNA in the chromatosome, the presence of H1 or H5 is not required for maintenance of the structure at 25 °C, since the circular dichroism spectrum of the chromatosome is only slightly affected by removal of the lysine rich histone.

While H1 or H5 is apparently not required for the maintenance of the structure of the additional DNA present in the chromatosome, thermal denaturation measurements indicate that the lysine rich histone stabilizes not only this additional DNA but also segments of DNA within the core particle. The thermal denaturation of core particles has recently been studied by spectrophotometric, circular dichroic, and calorimetric methods (Weischet et al., 1978); the data indicate that about 40 bp of DNA melt at a temperature of about 60 °C, while the remaining 100 bp of DNA are denatured at 74 °C, coincident with disruption of the protein nucleus of the core particle. Based in part on mapping studies which indicated that the 20 bp at each end of the core particle DNA is not tightly associated with histones (Simpson & Whitlock, 1976; Noll, 1977), Weischet et al. (1978) attributed the lower temperature transition to melting of 20 bp of DNA at each end of the DNA in the core particle.

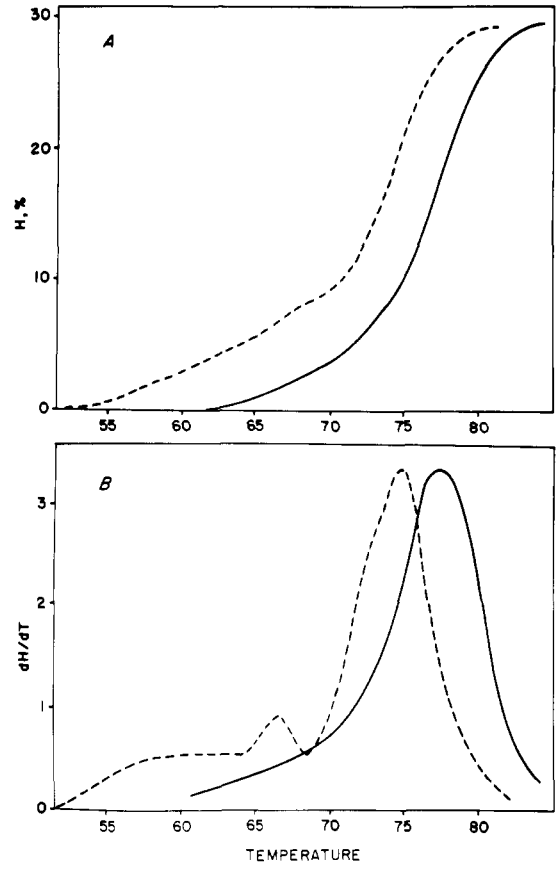

FIGURE 3: Thermal denaturation of chromatosomes and chromatosomes (−H1, −H5). Chromatosomes (—) or chromatosomes (−H1, −H5, (- - -)) in 0.25 mM EDTA, pH 7.0, were denatured as described in the Experimental Section. (A) Direct melting curve; H is the hyperchromicity, defined as the increase in A_{260} divided by the A_{260} at 35 °C. (B) Derivative melting curve.

In contrast to these findings with isolated core particles, the thermal denaturation of chromatosomes is (1) essentially monophasic and (2) shifted to higher temperatures. Although some tailing to lower temperatures is observed, the derivative melting curve for chromatosomes is nearly that expected for a single transition with a melting temperature of 77.5 °C (Figure 3). No melting is observed at the transition temperature for protein free DNA (45 °C in the solvent used); hence the additional DNA is stabilized by the presence of H1 or H5. No low melting transition comparable to that observed for core particles is present for the chromatosome; hence the 40 base pairs of DNA which denature early in the thermal disruption of core particle structure are also stabilized by the presence of the lysine rich histone. Finally, the increase in the major transition temperature for the chromatosome (Figure 3) vs. the core particle, coupled with the demonstration that core particle DNA denaturation accompanies disruption of histone secondary structure (Weischet et al., 1978), suggests that H1 or H5 may interact with the inner histones and/or the bulk of core particle DNA in a fashion which stabilizes the structure of the entire chromosomal subunit. Alternatively, H1 or H5 may affect melting of these DNA regions by a telostabilizing mechanism while interacting only with the terminal DNA regions.

Several changes in melting properties ensue when the lysine rich histone is removed from the chromatosome (Figure 3). The temperature for the major transition is lowered to 74 °C, similar to that for the isolated core particle. Additionally, two lower temperature transitions occur. The first is broad, cen-

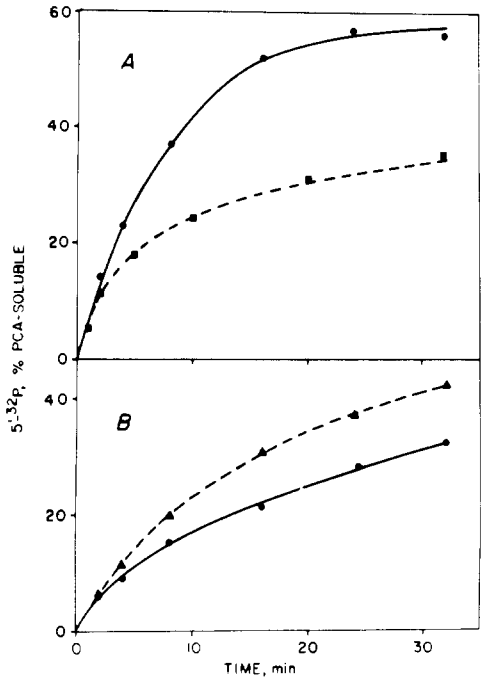

FIGURE 4: Kinetics of digestion of 5′-labeled chromatin particles by DNase I. (A) 5′-^{32}P-labeled core particles (—) or chromatosomes (- - -) were digested with DNase I at concentrations of 80 and 400 U/mL, respectively. Aliquots were removed at the indicated times and precipitated with perchloric acid in the presence of carrier DNA, and the fraction of the label made acid soluble was determined by scintillation counting. (B) 5′-^{32}P-labeled core particles (—) or chromatosomes (−H1, −H5) (- - -) were digested with DNase I at a concentration of 40 U/mL.

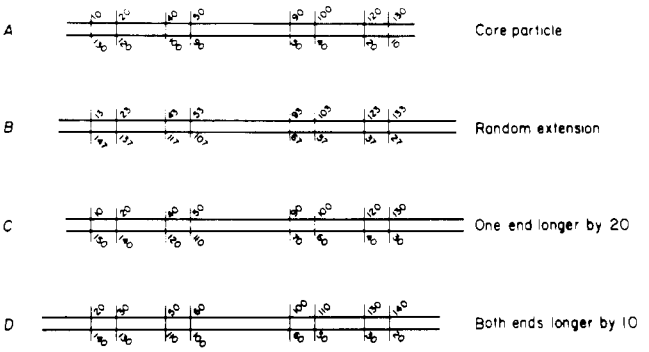

FIGURE 5: Hypothetical arrangements for the additional 20 bp of DNA in the chromatosome. (A) One hundred and forty base pairs of core particle DNA is shown diagrammatically, with the major DNase I cutting sites indicated by vertical bars. The numbers by each bar indicate the length of labeled DNA fragment expected in a mapping experiment using 5′-labeled DNA. (B) One hundred and sixty base pairs of DNA with 3 bp added to one end and 17 bp of DNA added to the other end of the core particle. (C) DNA formed by addition of 20 bp of DNA to one end of the core particle. (D) One hundred and sixty base pairs of DNA formed by addition of 10 bp of DNA to each end of the core particle segment.

tered at about 55 °C, and the second, sharper, is located at about 67 °C (Figure 3). The major transition likely corresponds to that observed at the same temperature for core particles; the two lower temperature transitions presumably derive from denaturation of the 60 bp of DNA which are not highly stabilized in the chromatosome (−H1, −H5) by interactions with the histone nucleus of the particle.

Both the melting data and the fact that the chromatosome contains 20 bp DNA more than the core particle, associated with the presence of the lysine rich histone, can be interpreted to suggest that this protein interacts with the ends of DNA in the chromatosome. This suggestion was evaluated by studies of the rate of digestion of 5′-^{32}P-end-labeled particles by DNase I. The rate of digestion of particles labeled at the DNA ends reflects the rate of cleavage by the nuclease at a site(s) less than 15 bases from the end, since fragments larger than this size would be precipitable by 10% perchloric acid and the digestion rate is assayed by the production of acid soluble radioactive label. Figure 4 compares the rate of digestion of such labeled chromatin particles for core particles, chromatosomes, and chromatosomes (−H1, −H5). When compared with core particles, chromatosomes are highly resistant to digestion of the terminal label; I estimate that 5–10 times as much DNase I is required to produce the same rate of digestion of chromatosomes as is necessary for core particles. In contrast, when H1 and H5 are removed from the chromatosome, the end label is digested more rapidly, by a factor of about twofold, than is the case for the core particle (Figure 4). Other data, not shown here, demonstrate that less marked, but still major, differences in the rate of digestion of total DNA by DNase I exist for core particles and chromatosomes. The rate of production of acid soluble A_{260} from chromatosomes is about 3–5 times slower than when core particles are digested. These observations could arise because the lysine rich histone interacted with both terminal regions of DNA in the chromatosome and some interior segments which are critical in the cleavage mechanism for degradation of particle DNA by DNase I. On the other hand, aggregated species formed under the digestion conditions may preclude access of the enzyme to its substrate by simple mechanical hindrance. This latter possibility receives support from recent studies of cavity sizes in precipitated oligonucleosome preparations (Burgoyne et al., 1978).

DNase I Cutting Sites in Chromatosomes and Core Particles. I have mapped the location of DNase I cleavage sites in the chromatosome using 5′-end label analyses as previously described (Simpson & Whitlock, 1976; Whitlock et al., 1977). Such mapping should be able to distinguish between several possible arrangements of the additional 20 bp of DNA in this particle vs. the core particle. Figure 5 shows several possible arrays for the additional DNA, each of which predicts different results in the mapping of cutting sites. If random lengths of DNA which are not multiples of 10 but together total 20 were protected by the lysine rich histone, the autoradiographic banding pattern which shows regularly spaced DNase I cutting sites located at multiples of ten from the 5′ end in the core particle should be largely obliterated in the chromatosome (Figure 5B). If 20 bp of DNA was added to one end of the core particle to form a chromatosome, the autoradiogram should be a combination of the normal core particle pattern, derived from one strand of the DNA, and the normal pattern shifted to longer fragment lengths by 20 nucleotides, from the opposite strand. This would have the general effect of retaining the distribution of fragments as multiples of ten, but leading to a more nearly equal cleavage site frequency distribution (Figure 5C). On the other hand, if ten base pairs of DNA was added to each end of the core particle DNA, a banding pattern like that observed for the core particle will be obtained, but the entire band distribution should be shifted to ten nucleotide longer fragments. (Figure 5D).

At the high concentration of DNase I necessary to map cutting sites in intact chromatosomes, we found autoradiograms like those observed with trypsin-treated core particles (Whitlock & Simpson, 1977) and degraded histones; hence the mapping experiments were performed on chromatosomes (−H1, −H5). Figure 6 shows an autoradiogram obtained with DNA isolated during digestion of chromatosomes (−H1, −H5) labeled at the 5′ end of the DNA; densitometric scans

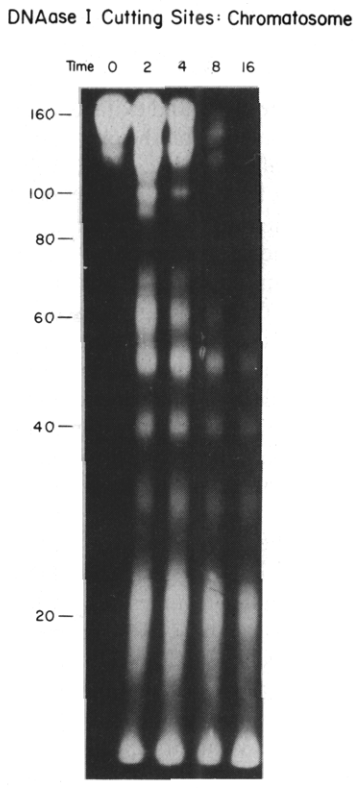

FIGURE 6: Autoradiogram of DNase I cutting sites in chromatosomes (−H1, −H5). Trace amounts of 5′-^{32}P-labeled particles were digested together with unlabeled core particles (A_{260} = 10) using 75 U/mL DNase I. Aliquots were removed, deproteinized, electrophoresed on denaturing 12% polyacrylamide gels, and subjected to autoradiography. Times of digestion are indicated above the several slots.

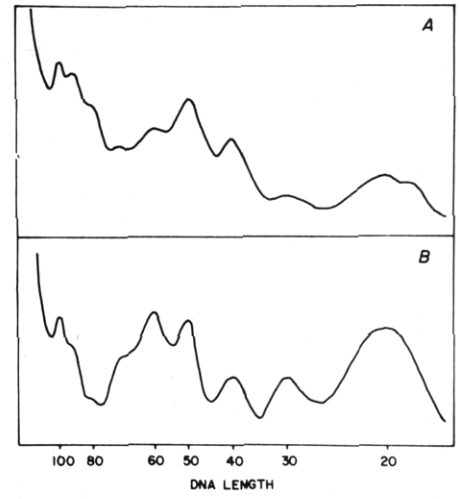

FIGURE 7: Scans of autoradiograms obtained during DNase I digestion of 5′-^{32}P-labeled chromatin particles. (A) Digestion of core particles for 4 min with DNase I. (B) Digestion of chromatosomes (−H1, −H5) for 2 min with DNase I.

of autoradiograms for digestion of core particles and chromatosomes (−H1, −H5) are shown in Figure 7. The core particle cleavage site distribution is quite similar to that shown previously for HeLa cell core particles (Simpson & Whitlock, 1976; Whitlock et al., 1977; Simpson, 1978); general features are preferred cleavage sites at 20, 40, 50, 90, 100, and 120 nucleotides from the 5′ end, limited cleavage at 30 and 110 bases from the ends, and a central valley with low frequency of DNase I cutting in the region 60–80 nucleotides from the ends of the DNA. In this central region, the band distribution obtained for these core particles is indicative of symmetry in distribution of cutting sites but differs from the patterns obtained with HeLa core particles; thus, sites 60 and 80 bases from the 5′ end are cut with intermediate frequency in chicken erythrocyte core particles and the site 70 bases from the end is highly resistant to DNase I (Figure 7A; cf. Figure 7A in Simpson, 1978). Whether this difference relates to length heterogeneity in the particles (unlikely), the presence of more nonhistone protein in the HeLa particles, or fundamental differences in interactions of DNA with the histone core in these two species remains moot.

Certain similarities to and some clearcut differences from the core particle pattern are present in the autoradiographic map of DNase I cleavage sites in the chromatosome (−H1, −H5) (Figure 6). First, in common with the core particle, DNase I cutting is detected at sites which are multiples of ten nucleotides from the end of the DNA in the chromatosome (Figures 6 and 7). This strongly suggests that the chromatosome is a defined structural entity in chromatin and does not arise merely from the random protection by the lysine rich histone of a total of 20 bp of DNA on the ends of the core particle. The overall digestion pattern for the chromatosome is like that of the core particle with ten nucleotides added to each of the fragments. This is suggested by the low frequency of cleavage at the 40 base site in the chromatosome when compared with the number of cuts made at the sites 50 and 60 bases from the end (compare with the similar distribution at 30, 40, and 50 bases for the core particle). Additionally, the central valley for the chromatosome occurs at 70–90 bases from the DNA ends, as opposed to 60–80 bases from the ends of the core particle (Figures 6 and 7). While I cannot define the site at 120 bases from the ends, predicted to be a low frequency site by this interpretation, it is apparent that the site 100 bases from the ends is a high frequency cutting site in the chromatosome, comparable to the site at 90 bases in the core particle. Two features of the digestion pattern are not predicted by the model in Figure 5D: low frequency of cutting at 30 and 110 bases from the 5′ end of the DNA in chromatosomes. They are also not predicted by any of the other models in Figure 5 and may reflect features of the organization of DNA in chromatin which are currently unknown. As a first approximation, however, these results suggest that the structure of the chromatosome may involve extension of the core particle DNA by addition of 10 bp more DNA to each end. Thus, the symmetry elements in the core particle may be maintained in the pattern of DNA organization in the chromatosome.

Discussion

A number of levels of chromatin organization have come under active scrutiny in the past few years, spanning a range from the role of particular proteins in metaphase chromosome structure (Paulson & Laemmli, 1977), on the one hand, to the intimate relationships of histones with one another and DNA in the core particle, on the other (see Kornberg, 1977; Felsenfeld, 1978). Here we add to this list a study of the physical properties of the chromatosome, a particle larger by 20 bp of DNA than the core particle and containing, in addition to the octamer of the four smaller histones, a molecule of lysine rich histone. As such, the chromatosome contains all the species necessary for the organization of the structure of relatively long segments of chromatin, making its structure of particular interest.

Two questions concerning the particle studied here deserve examination: its homogeneity and its origin. Others have noted heterogeneity in the monomer peak of micrococcal nuclease

digests of nuclei. Particularly, small amounts of (1) particles with DNA about 200 bp in length and two molecules of lysine-rich histone and (2) particles with DNA 160 bp in length but lacking H1 have been observed on nucleoprotein gels (Varshavsky et al., 1976; Todd & Garrard, 1977). I feel it likely that the chromatosome fraction is indeed a relatively homogeneous entity when prepared as described here. The gel scans and analytical protein determinations fit closely to values expected for a particle of 160 bp of DNA, an octamer of the inner histones and one molecule of H1 or H5. The nearly symmetrical nature of the melting of chromatosomes speaks to its homogeneity as does its behavior on analytical ultracentrifugation. Finally, particles of 160-bp length but lacking lysine rich histone are soluble in 0.1 M KCl and hence would not be included in the population studied here as chromatosomes.

Is the chromatosome an entity which is present in the nucleus, or could it arise as an artefact during the degradation of chromatin by micrococcal nuclease? For the latter case to be true, one would have to postulate that the bulk of H1 and H5 was released during degradation of chromatin and then a fraction of the lysine rich histone bound to chromatin particles. This seems unlikely. First, a number of separation techniques, performed at varying ionic strengths, have demonstrated the presence of approximately one molecule of lysine rich histone per chromatin subunit in nuclei (Olins et al., 1976), oligonucleosome fractions (Hozier et al., 1977; Noll & Kornberg, 1977), and a fraction of the 11S monomer peak in several different tissues (Varshavsky et al., 1976; Whitlock & Simpson, 1976a; Bakayev et al., 1977; Todd & Garrard, 1977; Noll & Kornberg, 1977). Second, it seems unlikely that released H1 or H5 should rebind selectively to particles with 160-bp length DNA as opposed to binding to 140-bp length DNA core particles, since other polycations can bind with facility to all of the 50% of DNA phosphates which are not complexed with histones (Clark & Felsenfeld, 1971; Simpson & Polacow, 1973). Third, lysine-rich histones can be annealed to chromatosomes (−H1, −H5) to create particles with physical properties similar to those of the chromatosome (D. Gruol & R. T. Simpson, unpublished observation). Finally, the discrete interactions defined in these studies for the lysine-rich histone with chromatosome DNA make it seem reasonable that this entity has been excised intact from the chromosome, much as is the core particle at a later stage of chromatin degradation. The compact nature of the chromatosome and the essentially monophasic thermal transition of this species suggest a precisely defined interaction of lysine-rich histones with DNA in the chromatosome. This conclusion is supported by studies of the mapping of DNase I cleavage sites in the chromatosome.

The additional DNA in the chromatosome appears to be placed in a precise fashion relative to the DNA of the core particle. Rather than H1 or H5 protecting a random length of DNA on each end of the core particle, totalling 20 bp, or protecting 20 bp of DNA on one end of the core particle, mapping data suggest that the chromatosome contains 10 bp of DNA at each end of the core particle. This arrangement would place the ends of the chromatosome DNA in phase with the DNase I cutting sites in the particle, and maintain the elements of symmetry in the digestion pattern. The two inconsistencies between the predicted (Figure 5D) and the observed (Figure 7B) patterns (see above) must be remembered in evaluating this model for arrangement of DNA in this particle.

The additional 20 bp of DNA in the chromatosome are sufficient for completion of two full terms of DNA around the histone nucleus of the chromatin subunit, using the known radius of distribution of DNA in the core particle, which indicates about 80 bp per turn (Pardon et al., 1975; Finch et al., 1977; Hjelm et al., 1977). Two full turns of DNA around a particle imply that the entry and exit sites for DNA ought to be located at the same point on the chromatin subunit, when viewed from the top of a cylindrical structure. Such an observation has been made by Wooley & Langmore (1977) in high resolution electron micrographs of chromatin containing H1. Entry and exit sites did not correspond when H1 was removed; the structure of chromatin also expands on removal of the lysine rich histone, leading to longer spacer regions between chromatin subunits. It seems likely that a molecule of lysine-rich histone could interact on the outside of the chromatosome with the DNA at each end of the nucleic acid; removal of this histone would decrease the stabilization of at least 20 bp of DNA, allowing chromatin decondensation and making the entrance and exit points for DNA in its path around the particle noncoincident.

The structure of the lysine-rich histones is appropriate for interaction with relatively long stretches of DNA in chromatin. In contrast to the smaller histones, which bear highly basic regions at their amino terminal ends, averaging about 25 amino acids in length, H1 has highly basic regions at both ends of the protein, some 20 amino acids near the amino terminal end and a long stretch of about 100 amino acids at the carboxyl terminus (data in Elgin & Weintraub, 1975). These highly basic regions are likely in an extended structure, while the central portion of the molecule may well be in a globular structure (Bradbury et al., 1975). One can easily envision either both ends of the carboxyl-terminal region of the lysine-rich histone interacting with the ends of chromatosome DNA, while the remainder of the protein molecule is folded, perhaps in a fashion which allows its interaction with other lysine-rich histones to provide the stabilization of the higher order coiling of the beaded chain of nucleoprotein. Such interactions are consistent with studies which show that H1 exists in a strucuture which allows cross-linking into large homopolymers (Chalkley & Hunter, 1975), and with electron microscopic investigations showing that nucleosomes with H1 can aggregate into 300Å fibers while similar particles lacking H1 do not do so (Finch & Klug, 1976).

It is interesting to speculate on the consequences of removing the lysine-rich histone from such a structure. Highly coiled chromatin would be sprung loose into a more extended conformation. An additional segment of the DNA would be available for cleavage by nucleases, particularly DNase I, which most readily cleaves the sites near the ends of core particle and chromatosome DNA (Simpson & Whitlock, 1976; Noll, 1977; Figures 6 and 7). In view of previous data which suggest (1) more rapid in vitro transcription of chromatin lacking H1 (Simpson, 1974a), (2) a more extended conformation for isolated chromatin lacking H1 (Polacow & Simpson, 1973; Simpson, 1974b) and for chromatin which is transcriptionally active in vivo (see Scheer, 1978, and references therein), (3) a lower melting temperature for chromatin lacking H1 (Reeck et al., 1972) and for chromatin in polytene chromosomes when puffed vs. the unpuffed state (Pages & Alonso, 1978), and (4) preferential susceptibility of transcribed genes to DNase I (Weintraub & Groudine, 1976; Garel & Axel, 1976), this seems not an unreasonable mechanism for conversion of a repressed gene segment to a transcriptionally active gene segment.

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