Volume 4 Number 9 September 1977 Nucleic Acids Research

Structure of transcriptionally-active chromatin subunits

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Received 24 June 1977

ABSTRACT

Rat liver chromatin is organized into regions of DNA which differ in degree of susceptibility to attack by the endonucleases DNase I and DNase II. The most nuclease-sensitive portion of chromatin DNA is enriched in transcribed sequences. This fraction may be separated from the bulk of chromatin by virtue of its solubility in solutions containing 2 mM $MgCl_2$. Both transcribed and nontranscribed regions of chromatin are organized into repeating units of DNA and histone, which appear as 100 Å beads in the electron microscope. The length of DNA in the repeat unit is the same for these two classes of chromatin (198 ± 6 base pairs in rat liver); however, the subunits of active, Mg^{++}-soluble chromatin differ from the nucleosomes of inactive regions of chromatin in several respects. Active subunits are enriched in nascent RNA and nonhistone protein and exhibit higher sedimentation values than the corresponding subunits of inactive chromatin.

INTRODUCTION

Methods for the purification of transcriptionally-active fractions of chromatin generally start with sonicated or pressure-sheared chromatin and fractionation is achieved by either density gradient sedimentation, column chromatography or buoyant density centrifugation (for review see Ref. 1). Since the work of Noll, Thomas and Kornberg (2) we are aware that mechanical shear damages native nucleosome structures, and hence fractionation results with sheared chromatin must be viewed with caution. In 1971 Marushige and Bonner (3) reported a fractionation scheme utilizing the endonuclease DNase II to cleave chromatin DNA. Fractionation is achieved in two ways: first, DNase II appears to attack transcribed regions of chromatin at a much higher rate than nontranscribed regions, and second, inactive chromatin can be removed from solution by precipitation with divalent cations, specifically Mg^{++}.

Many lines of evidence suggest that the nuclease-sensitive, Mg^{++}-soluble fraction of chromatin corresponds to transcribed regions of

chromatin *in vivo*. First, the amount of DNA recovered in this fraction is variable, but correlates with the transcriptional activity of the cell type under investigation (4). Second, fractionation is highly DNA sequence-specific. The DNA of the Mg^{++}-soluble fraction is a specific subset of the genome rather than a random sample of sequences and the active fractions of different cell types contain different populations of DNA sequences (5). Third, nascent RNA chains and RNA polymerase activity are copurified with the Mg^{++}-soluble chromatin fraction (3,6-8). Fourth, and most important, the DNA of the Mg^{++}-soluble fraction is enriched in sequences coding for both total cellular RNA (9) and for cytoplasmic messenger RNA (10). A specific mRNA sequence (globin) is preferentially localized in the active fraction of transcribing cells, but not in the active fraction of nontranscribing cells (10).

This report concerns the subunit structure of active chromatin. Active chromatin is organized in nucleosome-like structures containing about 200 base pairs of DNA in association with a full complement of histone protein. Active subunits differ from nontranscribed nucleosomes in their high content of RNA and nonhistone protein and in their sedimentation properties. Enzyme digestion studies suggest that active chromatin DNA is in a more open conformation than the DNA of nontranscribed chromatin regions.

MATERIALS AND METHODS

Preparation of nuclei and fractionation of chromatin. Rat liver nuclei were prepared by the method of Hewish and Burgoyne (11) and lysed by suspension in 0.2 mM EDTA (pH 7) as described by Noll *et al.*(2). Sodium acetate was added to a final concentration of 25 mM (pH 6.6). After various times of digestion with DNase II (Worthington, HDAC) at 2-10 enzyme units/ A_{260} unit of DNA, the pH of the sample was raised to 7.5 with 0.1 M Tris-HCl (pH 11). Chromatin was separated into a first supernatant (S1) and pellet (P1) by centrifugation (at 10,000 g for 10 min). The supernatant was further fractionated by the addition of $MgCl_2$ to 2 mM. The precipitate that forms is removed from solution by centrifugation (10,000 g for 10 min). The second pellet fraction is termed P2 and the final supernatant of Mg^{++}-soluble chromatin is termed S2.

HeLa cells were grown on the surface of 50-ml culture flasks in the presence of 10 - 20 ml of growth medium (Dulbecco's modified medium supplemented with 5% new-born calf serum). Cells were labelled with

^{3}H-uridine (25 Ci/mmole, Schwartz-Mann) at a concentration of 20 μCi/ml. After incubation for 1 h at 37° the medium was discarded; 10 ml of balanced salts solution was added and the cells were dislodged from the walls of the flask with a rubber policeman. The cells were lysed and fractionated into nuclei and cytoplasm (12). The nuclear pellet was suspended in 1 ml of 0.34 M sucrose-buffer A - 3 mM $CaCl_2$ (2), and chromatin was digested *in situ* with micrococcal nuclease (PL Biochemicals) at 200 units/ml for 4 min at 37°. The reaction was stopped by the addition of EDTA (pH 8) to 25 mM, and the nuclei were lysed as described (2).

Rat liver chromatin was also digested in intact nuclei with micrococcal nuclease (2) and with pancreatic DNase I (Worthington) (13).

Gel electrophoresis. DNA was purified from the various chromatin fractions by digestion with Proteinase K (Merck) at 20 μg/ml for 1 - 2 h at 37° in a buffer containing 0.5% SDS, 0.15 M NaCl, 0.05 M EDTA, 0.05 M Tris-HCl (pH 8). The DNA was extracted twice with chloroform-octanol (24:1, v/v) and precipitated with ethanol. After centrifugation to collect the precipitates, the pellets were dried under vacuum and then dissolved in gel buffer (0.2 M sodium acetate, 0.02 M EDTA, 0.04 M Tris-HCl, pH 7.8) containing 20% (w/v) sucrose. Electrophoresis of double-stranded DNA fragments was in 2% agarose gels. For estimation of single-strand DNA lengths, samples were denatured by boiling in 50% (v/v) formamide and electrophoresed in 10% acrylamide (0.15% NN'-methylene bisacrylamide) gels containing 7 M urea. Gels were stained with 0.5 μg/ml ethidium bromide and photographed.

Sucrose gradient sedimentation. Chromatin samples were centrifuged in 5 - 24% isokinetic sucrose gradients containing 10 mM Tris-HCl (pH 8). The gradients were formed according to Noll (14) with the following parameters: C_{TOP} = 5.1% (w/v), C_{RES} = 30.5% (w/v) and V_{mix} = 10.3 ml. Centrifugation was in the SW 40 rotor as described in the figure legends. Sucrose solutions were pretreated with diethylpyrocarbonate (0.05%, v/v), then heated at 60° for 12 - 18 h prior to use.

Analytical ultracentrifugation. Analytical ultracentrifugation was performed with an MSE Analytical Ultracentrifuge Mk. II, fitted with ultra-violet scanning optics (scanning the samples at 260 nm). All experiments were performed at 5° in 10 mM Tris-HCl (pH 8). Sedimentation coefficients were determined from the slope (fitted by regression) of plots of ln(r) against time, taking the radius of the midpoint of the boundary. These values were corrected for solvent viscosity and density,

using a partial specific volume calculated for the known composition of the particles of 0.66 ml/g, to give values of $S_{20,w}$. Molecular weights were determined by low speed sedimentation equilibrium. The attainment of equilibrium was ascertained by comparison of scans taken 24 h apart; when no difference could be seen the scan at the later time was taken to represent the equilibrium distribution. The data were analysed by determining the optical density at 40 to 60 points along each cell and calculating the slope of a plot of the logarithm of concentration against radius squared by regression analysis of sets of 11 consecutive points. A graph of the apparent weight average molecular weight against the concentration at the mid-point of each set of 11 data points was drawn and the weight average molecular weight at zero concentration calculated by extrapolation of 1/M against concentration. The partial specific volume was taken as 0.66 ml/g (as above) and the solvent density as 1.001 g/ml.

Electron microscopy. Samples for electron microscopy were dialyzed against 50 mM NaCl - 10 mM Tris-HCl (pH 8) and diluted to $1A_{260}$ unit per ml. Solutions were applied to carbon coated grids and stained with 1% uranyl acetate as described by Finch et al. (15). Microscopy was performed with a Phillips EM300 at 40 Kv at a magnification of 51,000 (146,000 magnification on photographic prints).

Analysis of chromatin composition. Histone and nonhistone protein and RNA content was determined as described by Bonner et al. (16).

RESULTS

Digestion of chromatin DNA with DNase II. A time course of digestion of chromosomal DNA in gently-lysed rat liver nuclei is presented in Fig. 1. After an initial rapid rise in the appearance of soluble material (S1), the rate of appearance falls progressively until about 70-80% of chromatin DNA is rendered soluble. When the first supernatant is fractionated into Mg^{++}-soluble (S2) and Mg^{++}-insoluble material (P2), we see that the soluble fraction forms part of the most nuclease-sensitive fraction of chromatin DNA.

Repeating units in Mg^{++}-soluble chromatin. The length of DNA in the Mg^{++}-soluble fraction of rat liver chromatin has been investigated previously by electron microscopy (4); the longest DNA fragments observed were about 2000 base pairs in length. On increasing times of digestion, the length of Mg^{++}-soluble chromatin DNA decreased progressively. Fig. 2 shows the results obtained when S2 DNA, isolated after various times of DNase II digestion is electrophoresed in 2% agarose gels. At early times

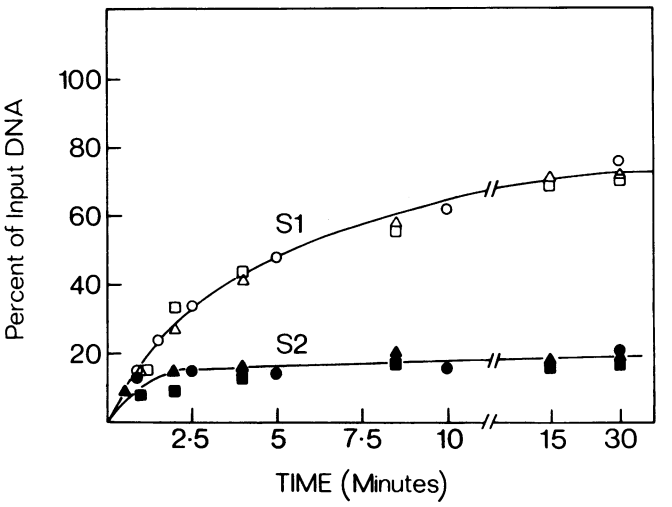

Figure 1. Time course of chromatin fractionation. Rat liver nuclei were lysed by suspension in 0.2 mM EDTA, pH 7, and sodium acetate was added to a final concentration of 25 mM (pH 6.6). After various times of incubation with DNase II (at 4 units/A_{260} unit of DNA), samples were withdrawn and added to an appropriate volume of 0.1 M Tris-Cl (pH 8) to give a final pH of 7.5. Separation of chromatin into the first supernatant fraction (S1) and subsequent fractionation into Mg^{++}-soluble (S2) and insoluble material was achieved as described. Aliquots of each fraction were taken for estimation of DNA content by absorption at 260 nm in 0.1 N NaOH. Open symbols, first supernatant (S1); filled symbols, Mg^{++}-soluble fraction (S2). Circles, squares and triangles represent independent experiments.

of digestion a typical band pattern is observed (11). A semi-logarithmic plot of distance of migration vs. log band number reveals that the higher hands are integral multiples of a unit length. As digestion proceeds, the DNA passes from multimers into a monomer band. The size of the monomer band appears to decrease with increasing times of digestion, presumably due to shortening of the DNA from the ends by further nuclease digestion (17).

To obtain accurate estimate of the DNA repeat length in S2 chromatin, DNA isolated after 1 min of DNase II digestion was co-electrophoresed with Hae II and Hae III restriction fragments of ØX174 RF DNA (Fig. 3). The lengths of the chromatin DNA fragments were determined from a least-squares regression analysis of the logarithm of restriction fragment length plotted against distance of migration. As the length of monomer DNA might be greatly affected by exonucleolytic attack, the repeat length was determined by taking the difference in length between successive multimer bands (17). From this analysis the DNA repeat is 199±6 base pairs, a value identical to the repeat length for a micrococcal nuclease digest of

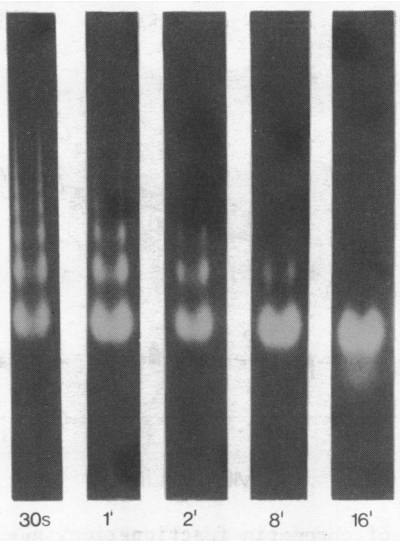

Figure 2. Agarose gel electrophoresis of Mg^{++}-soluble chromatin DNA. Rat liver nuclei were lysed and treated with DNase II (4 units/A_{260} unit) as described. At each of the times indicated an aliquot of the chromatin suspension was withdrawn and fractionated. Purified DNA from fraction S2 (approximately 5 - 10 µg per sample) was electrophoresed for 14 h at 24 mA in a 2% agarose gel. After electrophoresis the gel was stained with ethidium bromide (0.5 µg/ml) and photographed.

unfractionated nuclear chromatin (198±5 base pairs; Fig. 3). Thus it appears that the organization of Mg^{++}-soluble, transcriptionally-active chromatin DNA is similar to the organization of bulk transcriptionally-dormant DNA.

Nucleoprotein particles in Mg^{++}-soluble chromatin. Repeating subunits of nucleoprotein have been demonstrated by electron microscopy (15, 18-20) and by sedimentation of nuclease-treated chromatin in sucrose gradients (21). Electron micrographs of Mg^{++}-soluble chromatin reveal 100 Å beads which appear similar in size and shape to the nucleosomes of unfractionated chromatin (Fig. 4).

Noll (21) reports that monomer, dimer and trimer nucleosomes exhibit $S_{20,w}$ values of 11.2±0.4, 15.9±0.5 and 21.5, respectively (see also Fig. 6). The nucleoprotein subunits of Mg^{++}-soluble chromatin sediment somewhat faster than this (22). From 28 determinations similar to that shown in Fig. 5, the monomer subunit of Mg^{++}-soluble chromatin exhibits an $S_{20,w}$ of 14.0±0.8. The dimer sediments at 18.7±1.0S. The optical density observed at the top of the sucrose gradient depicted in Fig. 5 is due to light

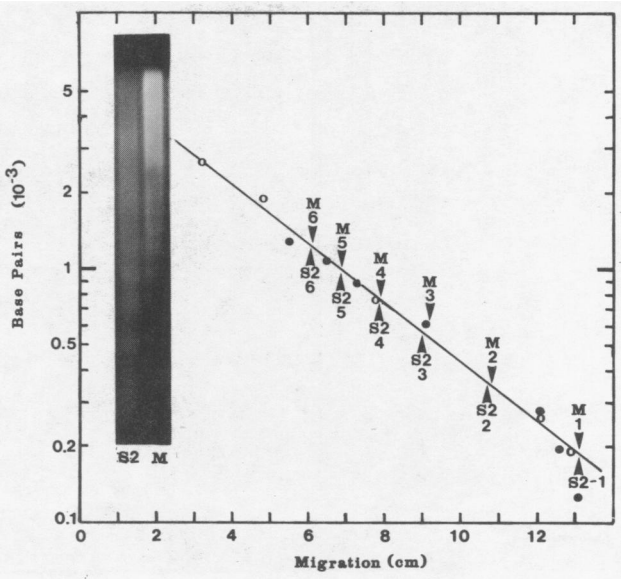

Figure 3. Determination of the size of DNA fragments from nuclease digestion of chromatin. Rat-liver nuclei were treated with DNase II (4 units/A_{260} unit for 1 min) and the chromatin was fractionated as described. Nuclei were also treated with micrococcal nuclease (100 units/ml for 3 min at 37°) with no subsequent fractionation. DNA was purified from the micrococcal nuclease-treated nuclei and from the Mg^{++}-soluble fraction (S2) of the DNase II-treated chromatin. Aliquots (10 µg) of each DNA sample were electrophoresed for 18 h at 30 mA in a 2% agarose gel. Sequenced restriction fragments (Hae II and Hae III) of ØX174 were used for size determination. (O) Hae II fragments; (●) Hae III fragments; M1-M6, position of micrococcal nuclease-generated chromatin DNA fragments, monomer through hexamer; S2 1-S2 6, position of DNase II-generated fragments of Mg^{++}-soluble chromatin DNA, monomer through hexamer. Gel: S2, Mg^{++}-soluble chromatin DNA produced by DNase II digestion; M, micrococcal nuclease digest.

scattering by nuclear debris; very little acid soluble material (3-5% of total DNA) is produced during there brief nuclease digestions. The sedimentation values reported above were obtained with isokinetic sucrose gradients (14) using 11S nucleosomes as a marker. If the 14S subunit of Mg^{++}-soluble chromatin has a different density from that of the 11S nucleosome, these $S_{20,w}$ values could be in error. On sedimentation in the analytical ultracentrifuge, 14S subunits of Mg^{++}-soluble chromatin exhibited an $S_{20,w}$ value of 15.4±0.3 (Fig. 7).

From a log-log plot of $S_{20,w}$ versus multimer number (Fig. 6), S values of Mg^{++}-soluble subunits are proportional to $M^{0.48}$. Noll and Kornberg (17) report that S values of micrococcal nuclease-generated

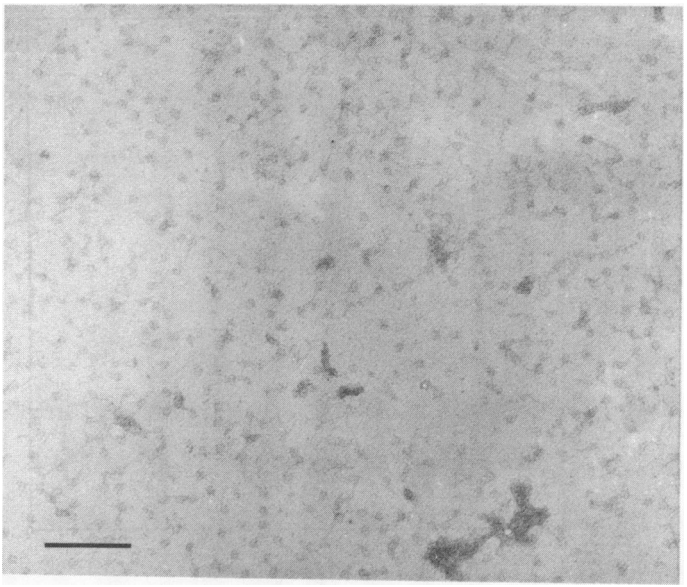

Figure 4. Electron micrograph of Mg^{++}-soluble chromatin. Rat liver chromatin was treated with DNase II (10 units/A_{260} unit) for 30 min and fractionated as described. The Mg^{++}-soluble fraction was run in a 5-24% isokinetic sucrose gradient to remove nuclear debris and acid-soluble material (see Fig. 5). Nucleoprotein subunits were collected from the gradient, dialyzed against 50 mM NaCl - 10 mM Tris-HCl (pH 8) and examined in the electron microscope. The bar represents 1000 Å.

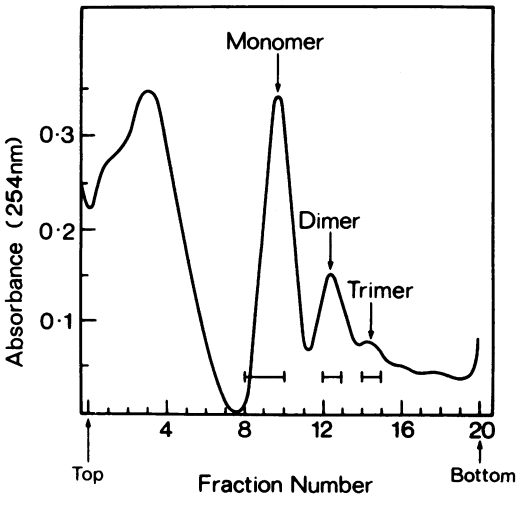

Figure 5. Sucrose gradient sedimentation of Mg^{++}-soluble chromatin. Rat-liver nuclei were prepared, treated with DNase II (10 units/A_{260} unit for 1.5 min) and the chromatin was fractionated as described. The Mg^{++}-soluble fraction (S2) was concentrated 3.3-fold with an Amicon Minicon device. 1.5 ml of this material was layered on a 5-24% (w/v) isokinetic sucrose gradient containing 10 mM Tris-HCl (pH 8). Centrifugation was at 35,000 rpm for 15 h in the SW40 rotor. The gradient was fractionated with an MSE device equipped with an Isco UV-absorbance monitor. Fractions were pooled as indicated.

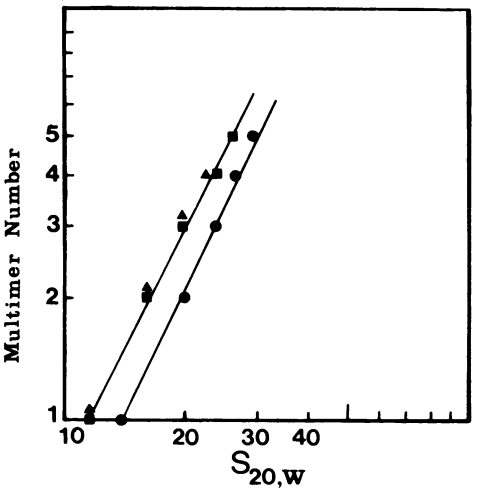

Figure 6. $S_{20,w}$ values of nucleosome multimers and subunits of Mg^{++}-soluble chromatin. Chromatin was treated with either DNase II (and fractionated as described) or with micrococcal nuclease (with no subsequent fractionation). Nuclease digests were sedimented in isokinetic sucrose gradients (Fig. 4) (●) Sedimentation values of subunits of Mg^{++}-soluble chromatin produced by DNase II cleavage. (▲ , ■) Sedimentation values of nucleosomes obtained after micrococcal nuclease treatment. (■ , data of ref. 18, ▲ this study).

nucleosomes are proportional to $M^{0.52}$. These data demonstrate that although the multimers of Mg^{++}-soluble subunits exhibit higher S values than nucleosome multimers, short chains of Mg^{++}-soluble subunits and nucleosomes have similar hydrodynamic conformations.

When DNA is extracted from the sucrose gradient fractions (Fig. 5) and electrophoresed in 2% agarose gels, the results of Fig. 8 are obtained. DNA isolated from the 14S peak is of monomer length; DNA from the 18S nucleoprotein peaks is of dimer length (with some monomer contamination). Trimer length DNA is obtained from the trimer subunit (along with contaminent monomer and dimer length DNAs).

The repeating unit in Mg^{++}-soluble chromatin contains 198 base pairs of DNA (Fig. 3); however, DNA isolated from 14S monomer subunits ranges from 160-200 base pairs in length, presumably due to partial exonucleolytic attack (17). The single strand length of 14S subunit DNA has been estimated by electrophoresis under denaturing conditions (Fig. 9). After brief (2 min) DNase II treatment monomer DNA ranges from 140 to 200 nucleotides in length. No discrete bands are seen at this stage of digestion. After longer times of DNase II treatment monomer DNA is reduced to 120 - 140 nucleotides. This length could represent the subunit core (17, 23 - 25). After very long digestion times (ca. 30 min), DNase II appears to attack within subunit cores giving rise to single strand fragments which are integral multiples of 10 nucleotides (slot C, Fig. 9, and ref. 26).

The chemical compositions of rat liver chromatin and chromatin

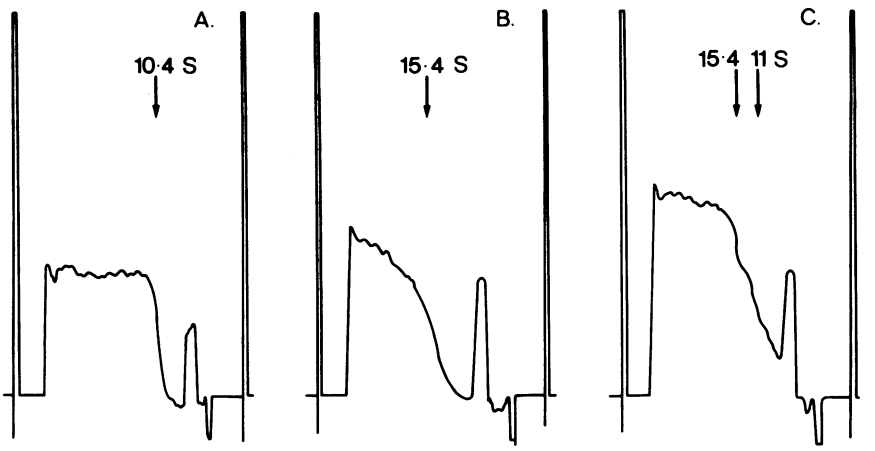

Figure 7. Sedimentation boundaries for particles in the analytical ultracentrifuge. (a) 'nucleosome' monomer produced by micrococcal nuclease digestion (nominally 11S); (b) '14S' particles; (c) partially degraded '14S' particles. Sedimentation (from right to left) was for approximately 30 min at 42750 rev/min in (a) and (b) and for approximately 40 min at 37700 rev/min in (c), giving comparable overall sedimentation. Arrows indicate position of boundary with sedimentation coefficient indicated.

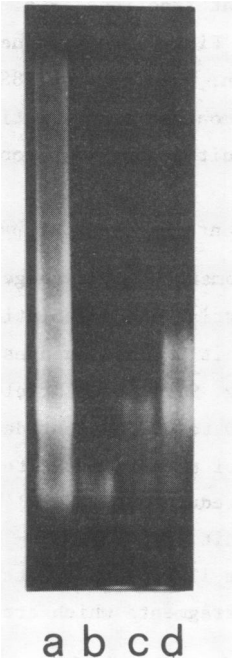

Figure 8. Agarose gel electrophoresis of DNA from sucrose gradient fractionation of Mg^{++}-soluble (S2) chromatin. Mg^{++}-soluble chromatin was prepared and centrifuged in an isokinetic sucrose gradient as described in the legend to Fig. 4. DNA was isolated from the fractions indicated. The purified DNAs were electrophoresed in a 2% agarose gel at 40 mA for 12.5 h. The gel was stained with ethidium bromide (1 μg/ml) and photographed. Slot a, DNA isolated from a micrococcal nuclease digest of rat-liver nuclei (300 units/ml for 1 min); Slot b, DNA from pooled fractions 9 and 10 of the gradient illustrated in Fig. 4; slot c, DNA from fraction 13; slot d, DNA from fraction 15.

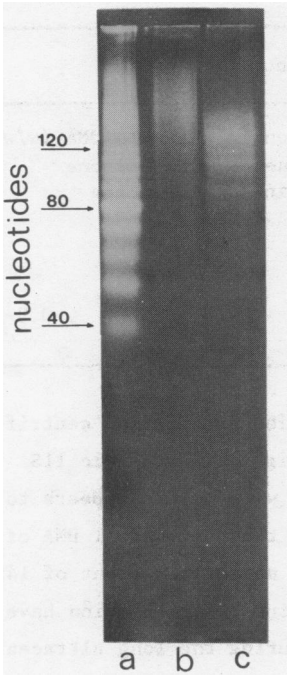

Figure 9. Estimation of single strand length of Mg^{++}-soluble chromatin monomer DNA. DNA was prepared from monomer subunits isolated from isokinetic sucrose gradients (Fig. 4). The DNA was denatured and electrophoresed at 35 mA for 14 h in a 10% acrylamide gel containing 7M urea. Slot a, standard pancreatic DNase I digest of rat liver nuclei (300 units/ml for 30 sec, ref. 24); slot b, Mg^{++}-soluble chromatin monomer DNA isolated after 2 min of DNase II treatment (at 10 units/A_{260} unit); slot c, Mg^{++}-soluble monomer DNA isolated after 30 min of DNase II treatment (at 5 units/A_{260} unit).

subunits are given in Table 1. Both 11S nucleosomes and 14S subunits contain a full complement of histone protein; furthermore, no species of histone is absent from the subunits of Mg^{++}-soluble chromatin when care is taken to prevent proteolysis (22). The major difference between Mg^{++}-soluble 14S subunits and 11S nucleosomes is the high concentration of nonhistone protein and RNA in the Mg^{++}-soluble subunits. Polyacrylamide gel electrophoresis reveals a complex pattern of proteins, suggesting that the subunits of Mg^{++}-soluble chromatin must be heterogeneous in composition (22). The heterogeneity may be reflected in the broadness of the sedimentation boundaries seen for 14S material when compared to 11S nucleosomes (see Fig. 7). Pederson and Bhorjee (8) have suggested that many of the non-histone proteins of Mg -soluble chromatin are RNA-binding proteins.

On occasion, sucrose gradient-purified 14S subunits exhibited $S_{20,w}$ values in the range 10.7 - 11.6S on sedimentation in the analytical ultracentrifuge. On occasion, two sedimenting components (11S and 14 - 15S) are observed (Fig. 7c). If 14S subunits are treated with RNase (10 µg/ml for 10 min at 37°) and then resedimented in an isokinetic gradient, the RNase-treater material sediments at 11S. Control 14S material resediments as before, at 14S. Furthermore, the 11S material obtained from RNase treatment of 14S subunits is insoluble in 2 mM $MgCl_2$. The 11S material produced by RNase treatment has a molecular weight of 190,000 - 209,000

Table 1. Chemical composition of chromatin subunits

	Composition relative to DNA (w/w)			
	DNA	Histone protein	Nonhistone protein	RNA
Unfractionated chromatin	1.00	1.06	0.65	0.05
11S nucleosomes	1.00	1.03	< 0.05	< 0.05
14S Mg^{++}-soluble subunits	1.00	0.97	1.35	0.3-0.4

daltons (as determined by low speed sedimentation equilibrium centrifugation) - a value identical to the molecular weight of authentic 11S nucleosomes. Thus the basis for fractionation with $MgCl_2$ appears to be the association of an RNA-protein complex with the nucleosomal DNA of active chromatin. Attempts at determining the molecular weight of 14S Mg^{++}-soluble subunits by sedimentation equilibrium centrifugation have failed due to the breakdown of this material during the long ultracentrifuge runs. The conversion of the 14S subunit to an 11S species is presumably due to contaminant RNase activity in either the chromatin sample itself or from the commercial DNase II used to prepare the chromatin subunits.

Purification of nascent RNA with 14S subunits. Bonner et al. (6) and Kimmel, Sessions and McCleod (7) have reported that the major portion of rapidly labelled RNA bound to chromatin is localized in the Mg^{++}-soluble fraction. To investigate the possibility that the RNA found in the 14S subunits of Mg^{++}-soluble chromatin might be newly transcribed RNA, the experiment of Fig. 10 was performed. HeLa cells were labelled in vivo with ^3H-uridine and nuclei were prepared. The nuclei were treated with micrococcal nuclease and the chromatin was analyzed in an isokinetic sucrose gradient. While the monomer nucleosome peak sediments at 11.5S, the first peak of radioactivity is at 13.7S. Similarly, the "multimers" of radioactivity sediment faster than the multimers of optical density. Micrococcal nuclease was used for this experiment as the commercial DNase II preparations have very high RNase activities. Under our conditions, DNase II exhibited significantly more RNase activity than did micrococcal nuclease, although the latter enzyme is a general nuclease rather than a specific DNase. Even so, the labelled RNA isolated from these gradients was quite small (4 - 6S in nondenaturing sucrose gradients). In the experiment

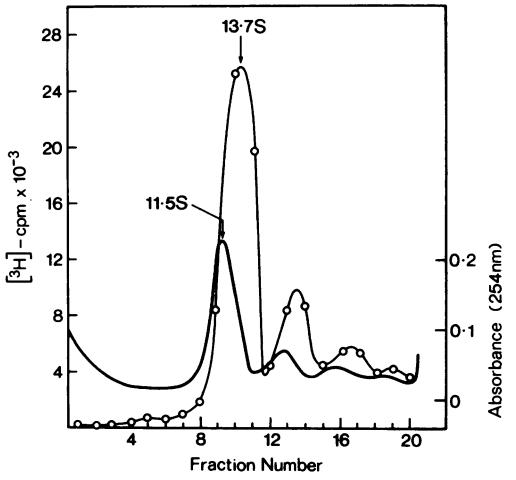

Figure 10. Sucrose gradient sedimentation of a micrococcal nuclease digest of ^{3}H-uridine-labelled HeLa nuclei. HeLa cells were labelled and nuclei were isolated as described. Nuclei were treated with micrococcal nuclease (200 units/ml for 4 min at 37°) and the reaction was stopped by the addition of EDTA (pH 8) to 25 mM. Chromatin was centrifuged in a 5 - 24% (w/v) isokinetic sucrose gradient at 35,000 rpm for 14.75 h. Aliquots of each fraction were precipitated with cold 10% trichloroacetic acid. Insoluble material was collected on GF/C filters and counted in Liquifluor. (——) Optical density profile; (-o-) trichloroacetic acid - insoluble radioactivity.

depicted in Fig. 10 no Mg^{++} precipitation step was performed prior to sucrose gradient sedimentation. If the "14S" region of the gradient (fraction 10, Fig. 10) is exposed to 2 mM $MgCl_2$ and then run in a second isokinetic gradient, the bulk of the nucleic acid sediments to the bottom of the centrifuge tube. However, about 50-70% of the radioactivity resediments at 14S, in association with 5-10% of the original input DNA. These data strongly suggest that nascent RNA chains are found in 14S subunits of Mg^{++}-soluble chromatin.

Fine structure of chromatin subunit DNA. Noll (27) has shown that digestion of chromatin with pancreatic DNase I generates a regular series of single-stranded DNA fragments which are integral multimers of a ten nucleotide repeat. It is thought that this repeat pattern reflects the ordered packaging of DNA about the histone core of the nucleosome (27 - 30). Is the DNA of actively transcribed chromatin organized in a similar manner? Fig. 11 shows that digestion of 14S subunits with DNase I produces a typical 10-nucleotide repeat. From densitometric measurements the same

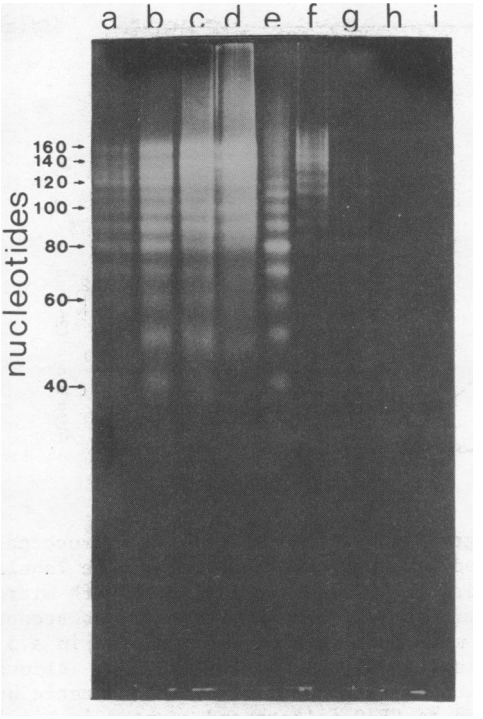

Figure 11. Electrophoresis of products of pancreatic DNase I digestion of chromatin monomers. Rat-liver nuclei were treated with either DNase II (1 min at 2.5 units/A_{260} unit) or with micrococcal nuclease (3 min at 300 units/ml) under standard conditions. The DNase II-treated chromatin was fractionated as described. Total nuclear lysates of the micrococcal nuclease-treated nuclei and the Mg^{++}-soluble fraction of the DNase II-treated chromatin were run on 5 - 24% isokinetic sucrose gradients. Chromatin monomers, recovered from the gradients, were digested with pancreatic DNase I at 30 units/ml in 10 mM $MgCl_2$ - 10 mM Tris. HCl (pH 8) at 37°. Reactions were stopped by pipetting aliquots into 1/5th volume of 0.5% sodium dodecylsulfate - 0.1 M EDTA (pH 8). The DNA was purified and concentrated by ethanol precipitation. The DNA samples were dissolved in 50% formamide - 40% sucrose in standard gel buffer and denatured for 5 min in a boiling water bath. Electrophoresis was for 16 h at 35 mA in a 10% polyacrylamide slab gel containing 7M urea. The gel was stained with ethidium bromide (1 µg/ml) and photographed. Slots a - d, products of DNase I digestion of total chromatin monomer (produced by micrococcal nuclease digestion); slot e, standard DNase I digest (300 units/ml for 30 sec) of rat-liver nuclei; slots f - i, products of DNase I digestion of Mg -soluble chromatin monomer. Digestion times: slots d and f, 30 sec; slots c and g, 1 min; slots b and h, 2 min; slots a and i, 5 min.

fraction of both 11S and 14S subunit DNA is found in the 10-nucleotide repeat. Thus a major fraction (ca. 85%, ref. 27) of 14S subunit DNA is converted into multimers of 10 nucleotides during DNase I digestion. The major difference between DNase I digestion of 11S nucleosomes and 14S

subunits is rate of enzymatic attack. Digestion of 14S subunits is much more rapid than digestion of 11S nucleosomes. The rate of DNase I digestion is most easily monitored spectrophotometrically, relying on the hyperchromicity of DNA at 260 nm (Fig. 12). Since hyperchromicity at complete digestion should be similar for all chromatin samples (i.e., it should be nearly that of naked DNA) the actual rates of change for the chromatin samples can be compared directly. The initial rate of digestion of Mg^{++}-soluble chromatin DNA is similar to or possibly faster than the rate of digestion of deproteinized DNA. Pellet fraction (P1) chromatin DNA is digested at about one-tenth this rate. These data are consistent with the results of Weintraub and Groudine (13) and Garel and Axel (31). These investigators report that active genes are preferentially attacked by DNase I. We note that both Mg^{++}-soluble chromatin and pellet fraction chromatin exhibit biphasic digestion patterns. Both samples have an initial "fast" phase of digestion followed by a transition to a slower rate of digestion. After 15 min of incubation neither sample has reached complete digestion.

If nuclei are first digested with DNase I and then fractionated according to the DNase II/Mg^{++}-solubility procedure, no material is recovered in the Mg^{++}-soluble fraction. Fig. 13 illustrates the result of this

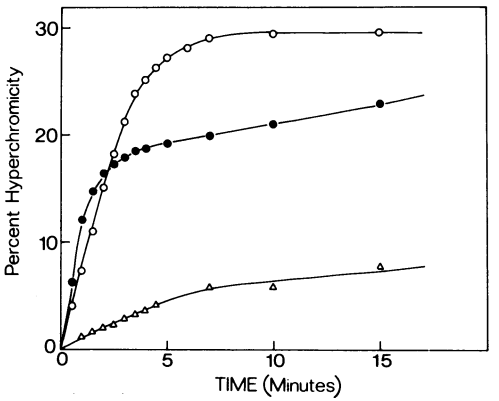

Figure 12. Kinetics of digestion of DNA and chromatin fractions with pancreatic DNase I. Rat-liver chromatin was treated with DNase II (10 units/A_{260} unit for 5 min) and fractionated as described. Chromatin fractions or purified DNA were digested with DNase I (100 units/ml, about 1 µg/ml) in 1 mM Tris - HCl (pH 8) - 10 mM $MgCl_2$. The A_{260} values of the substrate solutions were 0.5 - 0.7. The reaction was monitored spectrophotometrically at 260 nm. (O) DNA; (●) S2 chromatin; (Δ) P1 chromatin.

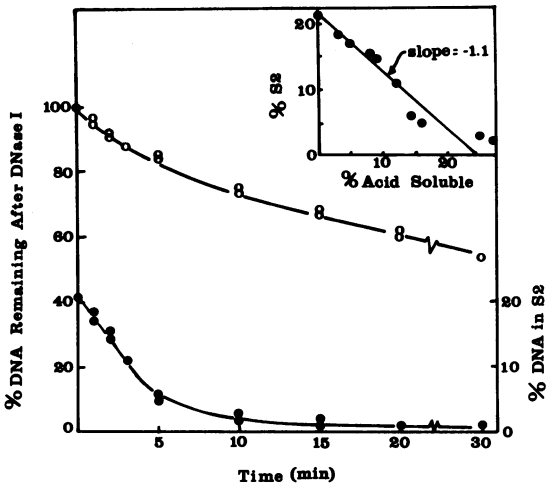

Figure 13. Effect of prior DNase I digestion of nuclei on recovery of Mg^{++}-soluble (S2) chromatin. Rat liver nuclei were prepared (11) and digested with DNase I (20 µg/ml) as described (13). At the times indicated, aliquots were withdrawn and pipetted into 5 volumes of 10 mM EDTA (pH 8). The nuclei were centrifuged and washed twice in 0.2 mM EDTA (pH 8). The pellet was resuspended in 0.2 mM EDTA and the nuclei were lysed as described (2). An aliquot of the nuclear lysate was taken for determination of the amount of DNA remaining after initial DNase I digestion. The remaining chromatin was treated with DNase II and fractionated as described. (O) Per cent of original nuclear DNA remaining after DNase I digestion. (●) Per cent of original nuclear DNA found in the Mg^{++}-soluble (S2) chromatin fraction after DNase II treatment and fractionation. Inset: Per cent of DNA recovered in S2 chromatin plotted against per cent of DNA rendered acid soluble by initial DNase I digestion.

experiment. After various times of treatment with DNase I aliquots were withdrawn and the reaction was stopped with EDTA. The nuclei were washed and then lysed; the lysate was treated with DNase II and the remaining chromatin was separated into Mg^{++}-soluble and insoluble fractions. After 5-10 minutes of DNase I treatment, about 20% of nuclear DNA is rendered acid soluble. Of the 80% of chromatin DNA remaining with the nuclei, none of this DNA can be recovered in the Mg^{++}-soluble fraction. The inset in Fig. 12 shows that the first 20% of nuclear DNA digested with DNase I is the same 20% of the original DNA which is isolated in the Mg^{++}-soluble fraction.

DISCUSSION

Repeating nucleoprotein subunits occur in both transcribed and non-

transcribed regions of chromatin. After digestion of chromatin with micrococcal nuclease, nontranscribed satellite DNA sequences (32) as well as messenger RNA-coding sequences (33 - 34) are recovered in monomer subunit DNA. Thus the mere association of DNA with histone is not sufficient to prevent transcription. The length of DNA and amount and types of histone per subunit appear to be the same for both active and inactive regions of chromatin. Moreover, subunits of both active and total chromatin appear as 100 Å beads in the electron microscope (Fig. 4). Many lines of evidence, however, demonstrate that the subunits of active chromatin are structurally different from the subunits or nucleosomes of nontranscribed regions. First, active genes are highly sensitive to attack by two nucleases, pancreatic DNase I (refs. 13 and 31, Figs. 11 and 12) and spleen DNase II (ref. 4 and Fig. 1). The experiment of Fig. 12 demonstrates that DNase I-sensitive and DNase II-sensitive regions are equivalent; prior treatment of nuclei with DNase I preferentially destroys the DNase II-sensitive, Mg^{++}-soluble fraction of chromatin. It is important to note that DNase I and DNase II act quite differently. DNase I destroys active genes (presumably by digestion within subunits) while DNase II preferentially cleaves active regions (by digestion between subunits; see Fig. 2). Second, thermal denaturation experiments show that transcribed DNA sequences are less tightly complexed with chromosomal proteins than nontranscribed regions (35 - 36). Third, the subunits of active chromatin exhibit higher sedimentation coefficients than the corresponding nucleosomes of inactive chromatin (Fig. 5). Finally, active subunits are enriched in nonhistone protein and RNA. Most of the newly labelled, chromatin-bound RNA sediments with the subunits of Mg^{++}-soluble chromatin. From the small size of this RNA and the chemical composition of active subunits, it is likely that each 14S subunit bears an RNA chain.

The basis for Mg^{++}-solubility of active chromatin appears to lie in the association of RNA or RNA-protein complexes with template DNA. RNase treatment of 14S active subunits converts these particles to an 11S species (the sedimentation value of monomer nucleosomes). This new particle has the same molecular weight as a nucleosome (equivalent to 140 base pairs of DNA plus 8 histones) and, like nucleosomes, is insoluble in 2 mM $MgCl_2$. At present we do not know whether alteration of nucleosome structure precedes genetic activity or is a consequence of it. Clearly further studies are needed to elucidate the structural differences between transcriptionally active and inactive regions of chromatin.

ACKNOWLEDGEMENTS

We gratefully acknowledge Drs. Francis Crick, John Gurdon, Aaron Klug, Ron Laskey and Ron Morris for advice and criticism, and Dr. J.T. Finch for performing the electron microscopy for us. One of us (J.M.G.) wishes to thank the Helen Hay Whitney Foundation for financial support.

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