Lysine-Rich Histones and the Selective Digestion of the Globin Gene in Avian Red Blood Cells†

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ABSTRACT: Deoxyribonuclease I (DNase I) is known to preferentially digest the adult globin gene sequences in avian red blood cells. We have investigated the contribution of histones H1 and H5 in maintaining the nuclease-sensitive structure about the globin genes. When the lysine-rich histones H1 and H5 were selectively removed from avian red blood cell nuclei, the rate of digestion with DNase I increased several fold. However, the globin genes in H1- and H5-depleted nuclei were still selectively digested. Since histone H1 is necessary for the higher order folding of the nucleosomes, these data suggest that DNase I recognizes an aspect of structural heterogeneity within each core particle rather than some higher order packaging of the nucleosome cores.

Chromatin is composed of beadlike subunit structures (nucleosomes) in which 170 to 210 base pairs of DNA wrap around an octomeric core of histones H2A, H2B, H3, and H4 (Kornberg, 1977; Felsenfeld, 1978; Elgin and Weintraub, 1975). The lysine-rich H1 histones are apparently attached to the DNA spacer regions between nucleosome cores (Nole and Kornberg, 1977; Kornberg, 1977) and condense the nucleosomes into higher order structures (Felsenfeld, 1978; Barrett, 1976; Finch and Klug, 1976; Renz et al., 1977).

Considerable evidence exists that actively transcribed regions have an altered structure throughout the transcriptional unit. One line of evidence for this comes from transcription experiments with isolated chromatin in which genes active in a particular tissue are selectively transcribed in vitro using exogenous bacterial polymerase (Barrett et al., 1974; Chiu et al., 1975; Axel et al., 1973; Gadski and Chae, 1978). Since *Escherichia coli* RNA polymerase would not be expected to recognize the eukaryotic promotors in chromatin, the tissue-specific transcription of chromatin with bacterial polymerase is likely to reflect some alteration in the conformation of active genes. Another line of evidence pointing to a special chromatin conformation in transcribed genes has come from the use of nucleases as probes of chromatin conformation. Active genes have been shown to be preferentially digested by DNase I (Weintraub and Groudine, 1976; Garel and Axel, 1976; Flint and Weintraub, 1977; Garel et al., 1977).

At the present time, it is not known what factors are responsible for the induction and maintenance of the altered conformation about active genes. It is possible that the greater availability of active genes to bacterial RNA polymerase or to DNase I reflects an altered higher order packaging of the nucleosomes within active genes. Experiments to directly test this possibility by checking whether the preferential DNase I digestion of active genes occurs with isolated nucleosomes have yielded conflicting results. Weintraub and Groudine (1976) found that in isolated nucleosomes from 18-day-old embryonic red blood cells globin genes were preferentially digested by DNase I. However, Garel and Axel (1976) found that DNase I did not preferentially digest the ovalbumin gene in isolated chicken oviduct nucleosomes. In view of these conflicting results, we felt that a different experimental approach might clarify the role of nucleosome higher order packaging in maintaining the altered chromatin conformation of active genes. Since histones H1 and H5 appear to be required for the higher order condensation of avian erythrocyte chromatin (Barrett, 1976; Cole et al., 1977), we have checked whether the preferential digestion of the globin genes in avian erythrocytes is sensitive to the removal of histones H1 and H5. We find that histones H1 and H5 are not required for the selective digestion of the globin genes by DNase I. These results suggest that the tissue-specific digestion of the globin genes reflects more than a mere perturbation in the packaging of the nucleosome cores.

Experimental Procedures

Materials. DNase I and staphylococcal nuclease were obtained from Worthington Biochemical Corp. [^{3}H]dATP, [^{3}H]dCTP, and [^{3}H]dGTP were obtained from New England Nuclear.

Isolation of Nuclei. Erythrocyte nuclei were isolated from 18-day-old chicken embryonic red blood cells as previously described (Weintraub and Groudine, 1976). Liver nuclei were prepared from chicken livers (excised from 18-day-old embryos) by the method of Axel et al. (1973).

Preparation of H1- and H5-Depleted Erythrocyte Nuclei. Erythrocyte nuclei were extracted with citric acid–phosphate buffers containing 10 mM NaH_2PO_4, 10 mM sodium citrate, 2 mM $MgCl_2$, 2 mM $CaCl_2$ (pH 3.0), and a series of salt concentrations ranging from 0.2 to 0.6 M. The nuclei were extracted for 20 min with the above buffer and then pelleted (1500*g* for 5 min in the HB-4 Sorvall head). This extraction was repeated once. Supernatants from both extractions were pooled and dialyzed against distilled water, and the proteins were allowed to precipitate overnight at −20 °C after adding 5 volumes of acetone. The extracted nuclear pellet was resuspended in 10 mM Tris[1]-HCl (pH 7.4), 10 mM NaCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$. The nuclei apparently remain intact through the citric acid extractions, since the DNA could be

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[1] Abbreviations used: Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; Temed, *N,N,N′,N′*-tetramethylethylenediamine; EDTA, (ethylenedinitrilo)tetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; nt, nucleotide.

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pelleted quantitatively in a low-speed spin (1500*g* for 5 min). Moreover, the acid-extracted nuclei appeared intact in the phase-contrast microscope.

Gel Electrophoresis of Histones. Nuclei or extracted supernatant proteins were dissolved in 2% sodium dodecyl sulfate ($NaDodSO_4$), 0.125 M Tris (pH 7.0), 0.5% β-mercaptoethanol, and 3 M urea. Nuclear samples were sonicated to shear the DNA. Electrophoresis was carried out on a 10-cm 18% polyacrylamide running gel containing 0.38 M Tris-HCl (pH 8.8), 0.1% $NaDodSO_4$, and 0.1% *N,N,N′,N′*-tetramethylethylenediamine (Temed). The 6% polyacrylamide stacking gel contained 0.125 M Tris-HCl (pH 6.8), 0.1% $NaDodSO_4$, and 0.2% Temed. The gels were run at 150 V for 7.5 h, stained with Coomassie blue containing 10% trichloroacetic acid and 25% 2-propanol, and destained with 7% acetic acid plus 10% ethanol. The destained gels were then dried and photographed.

Gel Electrophoresis of DNA. Electrophoresis of single-stranded DNA was performed on 7 M urea/8% polyacrylamide gels as described by Efstradiatis et al. (1975). Twenty micrograms of each DNA sample was heat denatured at 100 °C for 2 min in 7 M urea, loaded on a 20 × 20 cm slab gel, and electrophoresed at 300 V at room temperature. Gels were stained for 40 min in 1 μg/mL ethidium bromide and photographed under UV illumination.

Nuclease Digestion of Nuclei. DNase I digestion of isolated nuclei was performed in a buffer containing 10 mM Tris-HCl (pH 7.4), 2 mM $MgCl_2$, and 10 mM NaCl at a DNA concentration of 2 mg/mL. The nuclei were digested for 30 min at 37 °C with various concentrations of nuclease. The kinetics of the digestion reaction were monitored by determining the A_{260} absorbance that is soluble in 1 M $HClO_4$ plus 0.5 M NaCl. Erythrocyte nuclei were also digested with staphylococcal nuclease under identical conditions used for DNase I digestion.

To prepare preparative amounts of nuclease-resistant DNA, nuclei were digested with DNase I or staphylococcal nuclease until 10 to 20% of the DNA was rendered acid soluble. The nuclease-resistant DNA was then extracted with phenol–chloroform and chloroform–isoamyl alcohol (Weintraub and Groudine, 1976).

Preparation of Chicken Globin mRNA. White leghorn hens (2.0–2.5 kg of body weight) were made anemic according to the protocol of Williamson and Tobin (1977). Chickens were bled from the jugular vein, and the cells were washed three times in 0.14 M NaCl, 0.05 M KCl, 1.5 mM $MgCl_2$. The buffy coat, which contains most of the nonerythroid cells, was removed after each wash. Cells were lysed by suspension in 3 cell volumes of 25 mM NaCl, 5 mM $MgCl_2$, 2% Triton X-100, 1 mg/mL heparin (Sigma), and 25 mM Tris-HCl (pH 7.5). Nuclei and cell debris were removed by centrifugation at 10 000*g* for 10 min. The supernatant was then layered onto 6 mL of 60% sucrose, 25 mM NaCl, 5 mM $MgCl_2$, and 25 mM Tris-HCl (pH 7.5). Polysomes were pelleted by centrifugation at 50 000 rpm for 2.5 h at 4 °C in a Beckman 60 Ti rotor.

Pelleted polysomes were resuspended to 15 A_{260} units/mL in 0.5 M NaCl, 0.5% $NaDodSO_4$, and 10 mM Tris-HCl (pH 7.5) and heated at 65 °C for 2 min. Poly(A) mRNA was isolated using oligo(dT)-cellulose chromatography as described by Aviv and Leder (1972). Poly(A) mRNA was precipitated with ethanol and resuspended in 0.1 M NaCl, 1 mM EDTA, 0.5% $NaDodSO_4$, and 10 mM Tris-HCl (pH 7.5). This material was heated at 65 °C for 1 min and then sedimented through a 5–20% sucrose gradient made up in 0.1 M NaCl, 1 mM EDTA, 0.5% $NaDodSO_4$, and 10 mM Tris-HCl (pH 7.5). Centrifugation was at 26 000 rpm for 18 h at 25 °C in a Beckman SW27 rotor. The gradients were fractionated through a flow cell, and the 9S (globin mRNA) region was pooled and ethanol precipitated. This poly(A) mRNA has been shown to code for chicken globin polypeptides when translated in a wheat germ cell-free translation system (L. Lasky, unpublished observations).

Preparation of cDNA. Globin mRNA was transcribed using avian myeloblastosis virus RNA-dependent DNA polymerase (a gift of J. Beard) using a procedure modified from Efstradiatis et al. (1975). A 50-μL reaction mixture contained: 10 mM $MgCl_2$, 80 mM KCl, 50 mM Tris-HCl (pH 8.3), 40 μg/mL globin mRNA, 20 μg/mL oligo$(dT)_{12-18}$ (Miles), 20 μg/mL actinomycin D (Merck), 250 μM dTTP, [^{3}H]dATP, [^{3}H]dGTP, [^{3}H]dCTP, 20 mM β-mercaptoethanol, and 70 units of RNA-dependent DNA polymerase. The reaction was found to be completely primer dependent. After 20 min at 40 °C, the reaction mix was made 2% in $NaDodSO_4$, 10 mM EDTA, and 0.1 M NaOH. RNA was hydrolyzed for 10 min at 100 °C, after which the mixture was neutralized and passed over a Sephadex G-100 column. Fractions containing cDNA were pooled and precipitated with ethanol in the presence of 20 μg/mL yeast tRNA as carrier. The specific activity of the resultant cDNA was approximately 8×10^7 cpm/μg.

Large cDNA (larger than 300 nucleotides) was obtained by fractionation on alkaline sucrose gradients. The precipitated cDNA was dissolved in 0.1 M NaOH, 0.9 M NaCl, and 1 mM EDTA and centrifuged through a 5–20% sucrose gradient in the same buffer at 40 000 rpm for 20 h at 4 °C in a Beckman SW41 rotor. A 350-nt marker, the gift of Dr. Robert Goldberg, was run on a parallel gradient. cDNA larger than 300 nt in length were pooled, neutralized, and precipitated in the presence of 20 μg/mL yeast tRNA. The model single strand size of the globin cDNA was approximately 550 nt.

DNA Hybridization. The DNA samples (20 mg/mL) suspended in a mixture of 0.5 M NaCl, 80 mM Tris-HCl (pH 7.4), 0.1% $NaDodSO_4$, and 1 mM EDTA were heat denatured at 100 °C for 7 min and annealed at 65 °C to the labeled cDNA (2000 cpm/5 μL of reaction mixture). The globin probe had a specific activity of 8×10^7 cpm μg so that the hydridization was carried out with an excess of driver DNA to cDNA (1×10^7:1). Under these conditions, the calculated ratio of globin DNA to globin cDNA is 10:1. Polypropylene tubes overlaid with mineral oil were used for hybridization. At various times, a 10-μL sample of the hybridization mixture was pipetted into 230 μL of a mixture of 30 mM sodium acetate (pH 4.5), 0.15 M NaCl, 1 mM $ZnSO_4$, and 5 μg of denatured salmon sperm DNA. An aliquot (105 μL) of this mixture was immediately precipitated with Cl_3AcOH, while the other half was digested for 30 min at 37 °C with S1 nuclease (Sigma) before Cl_3AcOH precipitation. The S1 background radioactivity ranged from 1.3 to 3.0%.

Results

Removal of Histones H1 and H5 from Avian Red Blood Cell Nuclei. Histones H1 and H5 were extracted from intact erythrocyte nuclei using modifications of the method developed by Cole (personal communication) for depleting H1 histone from HeLa nuclei. By varying the ionic strength and pH of the extraction buffer, we attempted to optimize the selective extraction of histones H1 and H5 in high yield from intact nuclei. In order to maintain nucleosome integrity, none of the extraction buffers had a pH lower than 3.0. When red blood cell nuclei were extracted with various citric acid–phosphate buffers of pH 3.5 to 6.0, it was found that the salt concentrations necessary to extract H1 and H5 histones lysed the nuclei and severely reduced the yield of extracted nucleohistone. On

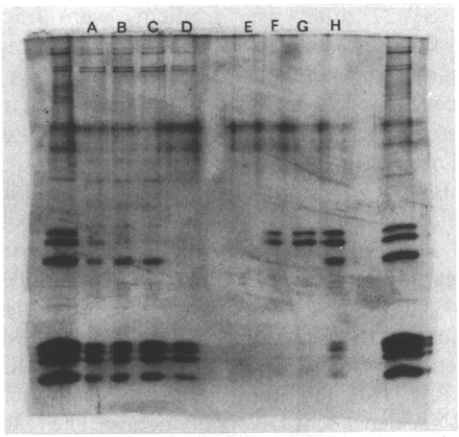

FIGURE 1: Extraction of lysine-rich histones from erythrocyte nuclei. Eighteen-day-old embryonic chicken erythrocyte nuclei were extracted with phosphate–citric acid buffers containing 0.2 (A), 0.3 (B), 0.45 (C), or 0.6 M (D) NaCl. The remaining nuclear proteins were solubilized in 2% $NaDodSO_4$, 0.125 M Tris (pH 7.0), 0.5% β-mercaptoethanol, and 3 M urea and resolved on a 15% $NaDodSO_4$–polyacrylamide gel. The 0.2, 0.3, 0.45, and 0.6 M NaCl extracts were electrophoresed on gel slots E–H. Electrophoresis of the proteins from control untreated nuclei is shown on the two end gel slots.

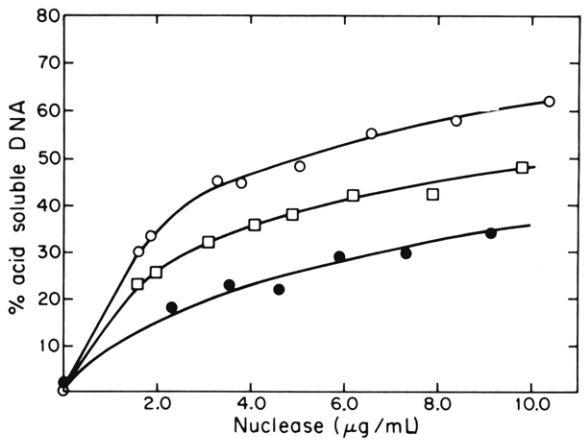

FIGURE 2: DNase I digestion kinetics of H1 and H5 histones depleted erythrocyte nuclei. Histone H1 (□) or histones H1 and H5 (○) were extracted from 18-day-old embryonic red blood cell nuclei. These preparations of nuclei along with untreated control nuclei (●) were then digested for 30 min with varying concentrations of DNase I. The release of acid-soluble DNA fragments was monitored using a Zeiss spectrophotometer.

the other hand, nuclei extracted with a citric acid–phosphate buffer of pH 3.0 remained morphologically intact (as judged by phase-contrast microscopy) even when exposed to 0.6 M NaCl. Figure 1 gives the results of $NaDodSO_4$ gel electrophoresis of the proteins extracted from erythrocyte nuclei using pH 3.0 buffers containing various salt concentrations and of the proteins remaining with the extracted nuclei. At 0.3 M NaCl, H1 histone is completely extracted along with a few of the nonhistone proteins. With a NaCl concentration of 0.6 M both H1 and H5 histones are extracted along with a small percentage of the core histones and a few nonhistone proteins.

Deoxyribonuclease I Digestion of H1- and H5-Depleted Nuclei. The effect of low-pH salt extraction on the kinetics of DNase I digestion of erythrocyte nuclei is shown in Figure 2. Avian red blood cell nuclei deficient in H1 histone had a 2.5-fold higher rate of DNase I digestion than did untreated nuclei. Nuclei depleted in H5 histone as well as H1 histone had a 4.5-fold greater rate of DNase I digestion.

It is possible that the observed increase in the rate of DNase I digestion of nuclei exposed to low-pH salt extraction may be unrelated to H1 and H5 histone extraction but, instead, reflects some change in chromatin conformation or an increased permeability of the nuclear membrane. To check for this, we have reconstituted the salt-extracted proteins back onto depleted nuclei using dialysis into low salt and then challenged these reconstituted nuclei with DNase I to determine their kinetics of digestion. Reconstituted nuclei always digested with kinetics identical to unextracted control nuclei (data not shown), which indicates that low-pH salt extraction does not lead to any irreversible changes in chromatin structure or membrane permeability. While these experiments do not rigorously rule out all possible alternative explanations, the data do suggest that removal of the lysine-rich histones H1 and H5 form avian erythrocyte nuclei increases the overall rate of DNase I digestion.

Weintraub and Groudine (1976) demonstrated that DNase I preferentially digests the globin genes in avian erythrocyte nuclei but not in avian liver nuclei. However, selective cleavage of the globin genes was not seen with staphylococcal nuclease. In order to ascertain what role histones H1 and H5 may play in maintaining the DNase I sensitive structure around globin genes in erythrocyte nuclei, we have tested whether DNase I preferentially digests the globin genes in erythrocyte nuclei which have been depleted of histones H1 and H5. To carry out these experiments, we first synthesized a complementary DNA (cDNA) probe to hemoglobin mRNA of anemic chicken reticulocytes using reverse transcriptase. Eighty percent of our globin cDNA probe hybridizes to purified globin mRNA with a $R_0t_{1/2}$ of 5.6×10^{-4}. We have used this globin cDNA to anneal with the DNA from various samples of nuclease-digested nuclei.

The globin cDNA probe was hybridized to a large excess of DNA from staphylococcal nuclease digested erythrocyte nuclei (Figure 3). The annealing of globin cDNA to sonicated DNA or to DNA from staphylococcal nuclease digested erythrocyte nuclei occurs with a $C_0t_{1/2}$ of approximately 500 to 600. In contrast, globin cDNA hybridizes to the DNA from DNase I digested erythrocyte nuclei with hybridization kinetics which are some four to six times slower. Thus, our results are consistent with the findings of Weintraub and Groudine (1976) that the preferential digestion of the globin genes in erythrocytes is seen with DNase I but not with staphylococcal nuclease.

When erythrocyte nuclei depleted of histone H1 are digested with DNase I and the extracted DNA is hybridized to globin cDNA, the kinetics of the annealing reaction are identical to that observed with hybridization reactions containing DNA from DNase I digested control nuclei (Figure 4). If nuclei depleted of both histones H1 and H5 are digested with DNase I, the kinetics of annealing of the extracted DNA to globin cDNA are again identical to that found with hybridization reactions containing DNA from DNase I digested control nuclei. These data strongly suggest that the DNase I sensitive structure about the globin gene is preserved in erythrocyte nuclei depleted of histones H1 and H5.

We have also checked whether DNase I preferentially digests globin genes in a tissue-specific manner. Chicken liver nuclei (from 18-day-old embryos) were digested with DNase I, and the nuclease-resistant DNA was isolated. The annealing of the DNA from DNase I digested liver nuclei and our globin cDNA probe reveals a $C_0t_{1/2}$ of around 500–600 (Figure 4), which is identical to the annealing rate of DNA from staphylococcal nuclease digested erythrocyte nuclei and our cDNA

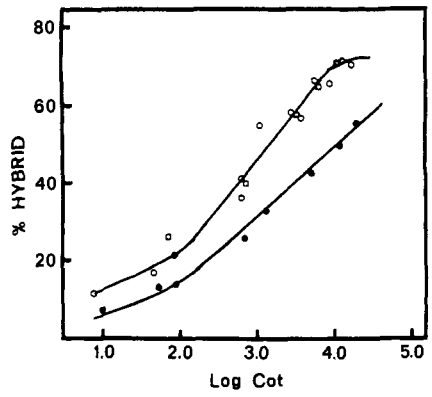

FIGURE 3: Kinetics of hybridization of globin cDNA to sonicated DNA or to the DNA isolated from nuclease-digested nuclei. Embryonic 18-day-old erythrocyte nuclei were digested with staphylococcal nuclease until 35% of the DNA was rendered acid soluble (□) or with DNase I until 20% of the DNA was acid soluble (●). The DNA from these nuclease-digested preparations was isolated and hybridized with globin cDNA. As a further control, DNA was extracted from untreated 18-day-old erythrocyte nuclei, sonicated to an average size of 100 base pairs, and then hybridized to the globin cDNA probe (○). The percentage of hybrid DNA was determined using S1 nuclease.

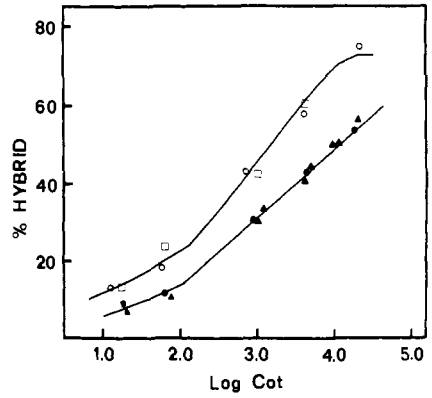

FIGURE 4: Kinetics of hybridization of globin cDNA to the DNA from DNase I digested nuclei depleted of lysine-rich histones. Erythrocyte and liver nuclei were prepared from embryonic 18-day-old red blood cells and excised liver: H1 histone depleted erythrocyte nuclei (●); H1 and H5 histone depleted erythrocyte nuclei (▲); H1 histone depleted liver nuclei (□); or untreated liver nuclei (○) were digested with DNase I until 20% of the DNA was rendered acid soluble. The DNA from these preparations was hybridized with the globin cDNA probe as in Figure 3.

probe (Figure 3). Thus, these results confirm Weintraub and Groudine's work that DNase I preferentially digests the globin genes in erythrocyte nuclei but not in liver nuclei.

Low-pH salt extraction of nuclei may alter the native structure of nuclei in such a way that later digestion of these nuclei with DNase I always generates DNA products with slower hybridization kinetics. To check for this possibility, we have prepared H1-depleted liver nuclei using the low-pH salt extraction procedure described for erythrocyte nuclei. As expected, DNA products from DNase I digested H1 histone depleted liver nuclei hybridized to our cDNA probe with the same kinetics as did whole sonicated DNA or DNA from DNase I digested complete liver nuclei (Figure 4). These data taken with our previous data demonstrate that DNase I digestion of H1-depleted nuclei generates DNA products which hybridize to globin cDNA with slow or rapid kinetics depending on whether erythrocyte or liver nuclei are used. We conclude that DNase I preferentially digests the H1 histone depleted nuclei of erythrocytes but not the H1 histone depleted nuclei of liver.

Since histones H1 and H5 depleted erythrocyte nuclei have a changed kinetics of DNase I digestion (Figure 2), it is possible that digestion of histones H1 and H5 depleted nuclei with DNase I generates a series of smaller DNA fragments than does digestion of intact nuclei. Thus, it is important to demonstrate that the reduced reassociation rate of globin cDNA to the DNA from DNase I digested erythrocyte nuclei depleted of H1 and H5 histones is not due to decreased DNA fragment size. To determine the size of the DNA fragments used in hybridization, the DNA samples were heat denatured and electrophoresed on a denaturing 8% polyacrylamide gel. In comparing the size distributions of each DNA sample in Figures 3 and 4, no correlation was observed between a sample's DNA fragment pattern on electrophoretic gels and its hybridization kinetics with globin cDNA (data not shown). Thus, we conclude that the reduced reassociation rate of globin cDNA to DNase I digested erythrocyte nuclei depleted of H1 and H5 histone is not due to a decreased size of the annealing fragments.

Discussion

That DNase I selectively digests active genes is perhaps the strongest line of evidence that transcribed genes have an altered chromatin structure. We have investigated the nature of the altered structure about the globin genes in avian erythrocytes by focusing on the possible role of histones H1 and H5 in maintaining the selective accessibility of DNase I for globin genes.

Cole (personal communication) has developed a low-pH procedure for removing H1 histone from intact nuclei. Using a combination of pH 3 and 0.6 M NaCl we have succeeded in removing the lysine-rich histones H1 and H5 from intact avian erythrocyte nuclei. Nuclei depleted of H1 histone have a 2.5-fold faster rate of DNase I digestion, while nuclei depleted of H1 and H5 histones have a 4.5-fold faster rate of digestion. Since we have ruled out any irreversible effects on DNase I digestion kinetics of the low-pH salt extraction procedure used to remove histones H1 and H5 from erythroid nuclei, our results suggest that the lysine-rich histones H1 and H5 have a marked effect on DNase I digestion kinetics. This is consistent with the findings of Silverman and Mirsky (1973) that removal of H1 histone from calf thymus nuclei greatly increases the rate of digestion with DNase I.

While removal of histones H1 and H5 from erythrocyte nuclei appears to markedly increase the rate of DNase I digestion, the preferential digestion of globin genes with DNase I is not affected. Partial digestion of histones H1 and H5 depleted erythrocyte nuclei with DNase I reduces the concentration of globin gene sequences in the remaining DNA by some 80%, as does DNase I digestion of whole erythrocyte nuclei. In contrast, partial DNase I digestion of either untreated or H1 histone depleted liver nuclei has no effect on final globin gene sequence concentration. The assay of globin gene sequence concentration is based on reannealing rates of globin cDNA with DNA isolated from digested nuclei. It is unlikely that the reannealing rates we have observed are an artifact of DNA fragment length, since no correlation was found between a sample's hybridization kinetics with globin cDNA and its size distribution as determined by gel electrophoresis. Thus, we conclude that histones H1 and H5 are not required for the maintenance of the altered DNase I sensitive conformation around the globin genes in chicken erythrocytes.

The lysine-rich H1 histone is the most variable histone in that qualitative and quantitative differences in H1 histone subfractions are observed in comparing different species or

even different organs within the same species (Elgin and Weintraub, 1975). H1 histone has long been associated with chromatin condensation (Littau, 1965). More recent data indicate that H1 histones are on the outside of the nucleosome core (Kornberg, 1977) and that H1 histone is required for the supercoiling of nucleosomes into solenoid-like structures (Renz et al., 1977; Finch and Klug, 1976). In the case of the nucleated avian red blood cells used in this study, the lysine-rich histones H1 and H5 appear to be involved in erythroid chromatin condensation (Cole et al., 1977). Thus, the lysine-rich histones appear to be required for the higher order folding of chromatin. However, the experimental results in the present study indicate that histones H1 and H5 are not required to maintain the altered DNase I sensitive structure about the globin genes in avian red blood cells. A reasonable conclusion from these data is that the preferential DNase I cleavage of the globin gene is related to some change in the structure of the globin gene nucleosome cores themselves rather than an altered packaging of the nucleosomes.

Weintraub and Groudine (1976) have shown that the globin genes in isolated erythrocyte nucleosomes can be preferentially digested by DNase I. In additional experiments, isolated erythrocyte nucleosomes were treated with trypsin which digests H1 and H5 histones along with the 20 to 30 amino acid residues from the N termini of the four core histones. The globin genes in these trypsin-digested nucleosomes were subsequently found to be preferentially digested by staphylococcal nuclease. From these two sets of data, Weintraub and Groudine concluded that the nucleosome cores themselves are altered in active genes. On the other hand, Garel and Axel (1976) found that the ovalbumin gene is no longer preferentially digested by DNase I when isolated nucleosomes are used, and they concluded from this that the higher order folding of the nucleosomes was involved in maintaining the active genes. Thus, these two sets of data are in apparent conflict. In attempting to obtain a better understanding of the above conflicting data, it perhaps should be noted that the isolation of nucleosomes requires cleavage with staphylococcal nuclease which may simply destroy the active gene conformation if the nuclease is allowed to degrade the DNA too far (e.g., a necessary chromosomal protein may be dislodged from the DNA during the digestion process with staphylococcal nuclease). It is, thus, imperative when comparing the nucleosomes from different tissues that the isolation of nucleosomes be carried out under standardized conditions. Alternatively, the observed descrepancy in the behavior of oviduct and erythrocyte nucleosomes to gene-specific DNase I digestion may reflect some trivial tissue difference. For example, oviduct nuclei could contain higher levels of proteolytic enzymes which could be active during the digestion with staphylococcal nuclease. In the present study, we have chosen an experimental approach to the study of the role of higher order nucleosome packaging in maintaining the active gene conformation which is free of any possible artifacts entailed in the isolation of nucleosomes. Our data confirm the results of Weintraub and Groudine (1976), indicating that the active gene nucleosome cores are in an altered conformation.

Any transcription unit must be composed of an array of many nucleosomes, which poses the question of how an altered nucleosome conformation is initiated and propagated within the transcriptional unit. It is important to note that, while H1 and H5 histones are not required for the maintenance of the active conformation, our data do not rule out an essential role for histones H1 and H5 (and, by implication, higher order packaging) in the initiation or propagation of an altered nucleosome conformation within the globin transcriptional unit.

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