Micrococcal nuclease and DNase I were used to study changes in the chromatin conformation of ovalbumin and globin genes during differential expression of these sequences. Oviduct nuclei, obtained from estrogen-treated chicks or chicks withdrawn from the hormone for 3, 4, or 5 days, were incubated with micrococcal nuclease until 1 to 3% of the DNA was rendered acid-soluble. The resulting DNA fragments then were separated into four size classes. In the stimulated oviducts, the concentration of the ovalbumin gene in mononucleosome-length DNA (165 to 200 base pairs) was 6-fold greater than in the fraction containing DNA fragments >1300 base pairs in length. This selective cleavage decreased progressively as a function of estrogen withdrawal time and correlated temporally with a decline in the concentration of oviduct nuclear estrogen receptors. The expressed globin genes in immature erythrocyte nuclei were also cleaved preferentially by micrococcal nuclease, whereas the transcriptionally silent globin sequences in mature erythrocyte nuclei were not.

The globin genes in both immature and mature erythrocyte nuclei were destroyed by DNase I 3 times faster than the greater part of the nuclear DNA. To determine if the globin genes in the mature erythrocyte were cleaved preferentially by DNase I, nuclei were incubated with this enzyme until 1 to 3% of the DNA was rendered acid-soluble. The resulting DNA fragments then were separated into four size classes. The concentration of globin genes in the size class containing the smallest DNA fragments (less than 300 base pairs) from both immature and mature erythrocyte nuclei was about 6-fold greater than in undigested DNA, and about 15-fold greater than in the fraction containing DNA fragments >1200 base pairs in length. These results suggest that the micrococcal nuclease sensitive conformation of the ovalbumin and globin genes in chromatin is dynamically related to the expression of these sequences. The DNase I sensitive structure as determined by both nucleolytic destruction and cleavage, in contrast, remains associated with globin sequences following their inactivation.

The structural organization of interphase chromatin is thought to play an important role in eukaryotic gene expression. The fundamental chromatin fiber is organized into repeating subunits called nucleosomes, each containing about 200 base pairs of DNA folded around a histone core (1). Although nucleosomes are apparently associated with transcriptionally active as well as inert regions of the genome, nucleosomal heterogeneity along the DNA fiber may allow for variation in higher levels of chromatin organization (2-4).

The differential folding of DNA along the chromatin fiber may represent an important mechanism in the control of differential gene activity. Condensation of specific chromosomal regions, whole chromosomes, or entire chromosomal complements during the differentiation of certain cells is commonly viewed as a mechanism for the inactivation of genetic expression (5-8). The generation of extended chromatin fibers from more compacted regions also occurs shortly before (9, 10) or at the time of (11) transcriptional initiation. The observations that the puffing patterns of dipteran polytene chromosomes are correlated with the appearance of specific transcriptional products during normal development or following hormone or heat-shock treatment (12-15) provide additional support for the proposal that transcription may be controlled, at least in part, by dynamic changes in the architecture of the chromatin compex.

Although the above microscopic evidence indicates a dynamic structural basis for differential gene activity, studies which have employed pancreatic DNase I as a probe for specific genes in chromatin have revealed that the chromatin conformation recognized selectively by this enzyme is maintained following cessation of transcriptional activity. Weintraub and Groudine (16) demonstrated that globin genes are selectively sensitive to digestion by DNase I in immature avian erythroid cells which are actively engaged in globin mRNA synthesis, as well as in mature erythrocytes in which globin mRNA synthesis is no longer observed. Similarly, changes in the concentration of globin mRNA sequences in mouse erythroleukemic cells and ovalbumin mRNA sequences in the chick oviduct are not accompanied by changes in the sensitivity of their DNA coding regions in chromatin to DNase I digestion (17-19). Studies by Garel et al. (20) have also indicated that genes which are transcribed at different rates are equally sensitive to selective destruction by DNase I. It, therefore, appears that the properties of genes in chromatin responsible for their selective digestion by DNase I are not directly related to the transcriptional process.

Previous studies from this (21) and another (22) laboratory have revealed that the ovalbumin gene in hen oviduct chromatin is in a conformation which is selectively cleaved by micrococcal nuclease. This conformation is related to transcriptional activity since the ovalbumin gene in liver and the globin genes in the oviduct, which are not engaged in RNA synthesis (22, 23), are not preferentially recognized by this nuclease (21, 22). In this report we have used micrococcal nuclease and DNase I to study potential changes in the chromatin of ovalbumin and globin genes during alterations in the expression of these specific sequences.
**EXPERIMENTAL PROCEDURES**

Animals—Four-day-old female chicks were injected with 1 mg/day of estradiol-17β (10 mg/ml of sesame oil) for 14 days and then withdrawn from the hormone for the times indicated in the figure legends. Oviduct tissues were fixed, sectioned, and stained as described previously (24).

Anemia was induced in adult hens by injection of 1 ml of 1% neutralized phenylhydrazine daily for 6 days. Blood cells were collected by cardiac puncture on the 7th day into phosphate-buffered saline (Gibco), containing 0.01% heparin. Cell types were identified by light microscopic examination of brilliant cresyl blue-stained preparations as described by Ringerz and Bolund (25). This immature erythrocyte population was composed of about 45% early and mid-polychromatic erythrocytes, about 54% late polychromatic erythrocytes, and less than 2% mature erythrocytes. Blood from normal hens contained >98% mature erythrocytes.

Preparation and Digestion of Nuclei—All steps were carried out at 4°C unless otherwise stated. Nuclei were prepared from laying hen or chick oviducts as described by Bloom and Anderson (26) and from mature or immature erythrocyte nuclei as described by Weintraub and Groudine (18). Nuclei were washed in digestion buffer (0.35 M sucrose, 10 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM MgCl₂, 0.5 mM CaCl₂), centrifuged at 10,000 x g for 5 min, and resuspended in the same buffer at a concentration of 40 A₂₆₀ units/ml. Nuclei were either digested at 0°C by addition of DNase I (EC 3.1.4.5) (10 μg/ml) for 4 min or warmed to 37°C for 3 min and digested by the addition of micrococcal nuclease (EC 3.1.4.7) (75 units/ml) for 1.5 to 10 min. Nuclei were incubated at 37°C for 30 min at a concentration of 3 A₂₆₀ units/ml of 0.25 M sucrose, 10 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM MgCl₂, containing [³H]estradiol (1 to 10 x 10⁻⁶ M) or [³H]estradiol (1 to 10 x 10⁻⁸ M) in the presence of a 100-fold excess of diethylstilbestrol. Specific binding was calculated by subtracting nonspecific binding (the [³H]estradiol present in aliquots containing a 100-fold excess of nonlabeled diethylstilbestrol, from total bound [³H]estradiol.

**Nuclear Estrogen Receptors**—Nuclear estrogen receptors were quantified by the [³H]estradiol exchange assay as described by Anderson et al. (27) in aliquots of the oviduct nuclear suspensions. Nuclei were incubated at 37°C for 30 min at a concentration of 3 A₂₆₀ units/ml of 0.25 M sucrose, 10 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM MgCl₂, containing [³H]estradiol (1 to 10 x 10⁻⁶ M) or [³H]estradiol (1 to 10 x 10⁻⁸ M) in the presence of a 100-fold excess of diethylstilbestrol. Specific binding was calculated by subtracting nonspecific binding (the [³H]estradiol present in aliquots containing a 100-fold excess of nonlabeled diethylstilbestrol) from total bound [³H]estradiol.

Preparation of DNA—Nuclei were made 1 M NaCl, 1% SDS and deproteinized by two extractions with chloroform/isoamyl alcohol (24:1). The DNA was ethanol-precipitated and dissolved in 5 mM EDTA (pH 7.0). DNA from undigested nuclei or from the digested samples indicated in the figure legends was sonicated using six 20-s bursts with intermittent cooling, to an average single-stranded length of ~200 nucleotides, as determined by denaturing alkaline agarose gel electrophoresis (28). Following sonication, samples were made 0.3 M NaOH, 0.1% SDS and incubated at 37°C for 18 h to hydrolyze the DNA and dissolved in 5 mM EDTA.

DNA-cDNA Hybridization—The preparation and characterization of ovalbumin and globin mRNAs and their respective cDNAs has previously been reported (21). Moderate cDNA excess hybridizations were performed as described by Bloom and Anderson (21). Estimates of the relative concentration of ovalbumin genes in hen oviduct DNA determined by moderate cDNA excess reactions were essentially identical to corresponding estimates obtained from DNA sequence excess experiments (21). The quantity of cDNA hybridized was expressed as the percentage of total acid-precipitable radioactivity in the sample.

**Effect of Estrogen Withdrawal on the Specificity of Micrococcal Nuclease for the Ovalbumin Gene in the Chick Oviduct**—The regulation of ovalbumin mRNA synthesis by steroid hormones in the chick oviduct represents a developmental system which is well suited for the study of the
micrococcal nuclease (Fig. 2), therefore, does not result from a loss of oviduct cells capable of ovalbumin production. The progressive decline in the ability of micrococcal nuclease to cleave selectively the ovalbumin sequences after estrogen withdrawal (Fig. 3A) was paralleled by a decrease in the concentration of estrogen receptors in oviduct nuclei (Fig. 3B). The level of estrogen receptor sites decreased from about 4,000 sites/nucleus in the stimulated oviducts to about 300 sites/nucleus in oviducts obtained from chicks withdrawn from the hormone for 6 days. Longer withdrawal periods did not result in a further decline in nuclear estrogen receptor (data not shown).

Specificity of Micrococcal Nuclease for Globin Genes during the Terminal Stages of Erythroid Cell Maturation—The experiments presented above (Figs. 1 to 3) indicate that the selective sensitivity of ovalbumin coding DNA in chromatin to micrococcal nuclease may reflect a structural feature which is related to the transcriptional activity of the ovalbumin gene. If such a relationship represents a generalized phenomenon, the transcriptional inactivation of globin genes during the terminal stages of avian erythropoiesis (25) should be accompanied by a decrease in the selective nuclease cleavage of the globin genes in erythroid chromatin. To examine this possibility we have characterized the nuclease sensitivity of the globin genes in nuclei prepared from immature (polychromatic) erythrocytes, which are actively engaged in globin mRNA synthesis, and from mature erythrocytes in which chicks as determined by histological analysis (not shown). The decline in the sensitivity of the ovalbumin sequences to micrococcal nuclease (Fig. 2), therefore, does not result from a loss of oviduct cells capable of ovalbumin production.

Fig. 3A shows the relative concentration of ovalbumin genes in the four DNA size classes prepared from estradiol-stimulated chicks, and chicks withdrawn from the hormone for 3, 4, and 6 days. By 3 days, there was a 70% reduction in the nuclease sensitivity of the ovalbumin gene and by 6 days, the concentration of ovalbumin coding sequences in the four DNA size classes was similar to total undigested nuclear DNA. The rate of ovalbumin mRNA synthesis also declines rapidly during the first 3 days after cessation of estrogen injection (23). The progressive decline in the ability of micrococcal nuclease to cleave selectively the ovalbumin sequences after estrogen withdrawal was paralleled by a decrease in the concentration of estrogen receptors in oviduct nuclei (Fig. 3B). The level of estrogen receptor sites decreased from about 4,000 sites/nucleus in the stimulated oviducts to about 300 sites/nucleus in oviducts obtained from chicks withdrawn from the hormone for 6 days. Longer withdrawal periods did not result in a further decline in nuclear estrogen receptor (data not shown).

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Conformational Changes of Genes in Chromatin

FIG. 4. Distribution of the globin gene in different size DNA fragments. Nuclei from immature erythrocytes (A) and mature erythrocytes (B) were digested with micrococcal nuclease (75 units/ml) for 1.5 min. The DNA was extracted from the digested nuclei and separated into four molecular weight size classes as described in Fig. 1. The two larger molecular weight size classes were sonicated as described in Fig. 2. DNA (0.375 mg/ml) from each size class was annealed to 3.4 x 10^4 cpm/ml of globin cDNA for the times indicated. The DNA/cDNA ratio employed resulted in 9% hybridization at saturation with total undigested DNA. The DNA size classes 1 (M), 2 (M), 3 (X-X), and 4 (O-O) for both A and B contained approximately 2, 5, 15, and 78% of the DNA, respectively, and were essentially identical to those shown in Fig. 1.

Globin mRNA synthesis has ceased. Erythroid nuclei were digested with micrococcal nuclease (75 units/ml for 1.5 min) and the resulting DNA fragments were separated into four size classes as described in Fig. 1. Hybridization of globin cDNA to the DNA size classes prepared from immature erythrocyte nuclei revealed that the globin genes were selectively cleaved by this nuclease (Fig. 4A). The concentration of globin coding sequences in monomer length DNA was about 3-fold greater than in undigested DNA and 6-fold greater than in the fraction containing DNA fragments >1300 base pairs. A reduction in the nuclease sensitivity of the globin genes was observed in mature erythrocyte nuclei since the concentration of globin sequences in each size class was similar to that in undigested DNA (Fig. 4B). Bellard et al. (22) have also recently shown that the globin genes in the mature erythrocyte nuclei are not cleaved preferentially by micrococcal nuclease.

To study further the nuclease sensitivity of the globin genes during erythroid maturation, similar studies were performed at later times in the digestion when more of the nuclear DNA had been converted into monomer size fragments. Fig. 5 shows the concentration of globin sequences in the four DNA size classes obtained from immature and mature erythrocyte nuclei which were digested with micrococcal nuclease (75 units/ml) for 1.5, 5, or 10 min. The concentration of globin genes was inversely related to the length of the DNA fragments derived from immature erythrocyte nuclei at all times in the digestion. Enrichment of globin sequences in monomer length DNA was greatest early in the digestion when the quantity of monomeric DNA was at a minimum. As the amount of DNA in the monomer size class increased with increasing digestion time, there was a decrease in the concentration of globin sequences in this fraction. After a more extensive digestion (450 units of micrococcal nuclease/ml for 15 min; data not shown), when >80% of the DNA fragments were of monomer length, the concentration of globin genes was the same as in total nuclear DNA. This result, which is in agreement with an earlier report (16), shows that the globin genes were not selectively degraded by micrococcal nuclease digestion of immature erythrocyte nuclei. The concentration of globin coding sequences in the four size classes prepared from mature erythrocytes was similar to the concentration of these sequences in undigested chicken DNA regardless of digestion time. These results support our previous conclusion that globin genes are preferentially cleaved by micrococcal nuclease in the immature erythrocyte nuclei as compared to the bulk of the nuclear DNA and, more importantly, as compared to the globin genes in the mature erythrocyte nuclei which are transcriptionally silent.

Specificity of DNase I for Globin Genes during the Terminal Stages of Erythroid Cell Maturation—The results

FIG. 5. Quantitation of the globin gene sequences in different size DNA fragments with increasing micrococcal nuclease digestion. Nuclei from immature or mature erythrocytes were digested with micrococcal nuclease (75 units/ml) for the indicated times. Following digestion, the DNA was separated into four molecular weight size classes and prepared for hybridization analysis as described in Fig. 3. DNA (0.375 mg/ml) from each size class was annealed with 3.4 x 10^4 cpm/ml of globin cDNA as in Fig. 4, and the relative concentration of globin genes in each DNA size class was determined as described in Fig. 3. The DNA size classes 1 (O-O), 2 (O-O), 3 (X-X), and 4 (X-O) for both immature and mature erythrocytes are in order of increasing molecular weight and are similar to those shown in Fig. 1. The percentages of DNA in size classes 1 to 4 of immature erythrocytes at 1.5 min of digestion were 2, 5, 12, and 80; at 5 min of digestion, they were 4, 9, 25, and 62; and at 10 min of digestion, they were 11, 25, 53, and 9, respectively. Similar percentages of DNA were obtained in size classes 1 to 4 of mature erythrocytes at 1.5, 5, and 10 min of digestion.
presented above (Figs. 1 to 5) suggest that the chromatin conformation recognized selectively by micrococcal nuclease is correlated with transcriptional activity. Several investigators have indicated, however, that the preferential sensitivity of specific genes in chromatin to pancreatic DNase I digestion is independent of their transcriptional rates (16–20). To determine if this apparent difference is biological or methodological in nature, we have reinvestigated the relationship between the transcriptional activity of the globin genes in erythroid cells and the sensitivity of these specific sequences to DNase I.

Weintraub and Groudine (16) have clearly shown that the digestion of about 15% of the DNA in nuclei with DNase I preferentially destroys globin genes in chick erythroblasts which synthesize globin mRNA, as well as in mature erythrocytes which do not. It is possible, however, that the rate of digestion of globin genes by DNase I might be dependent on their transcriptional activity. Fig. 6 shows a comparison of the relative rates of digestion of the ovalbumin and globin genes by DNase I in immature and mature erythrocyte nuclei. The globin coding sequences are destroyed about 3 times faster than the greater part of the nuclear DNA in both erythroid cell types. That this preferential destruction of the globin genes by DNase I is related to the transcriptional history of these specific sequences is indicated by the observation that the digestion of the ovalbumin genes paralleled the digestion of the bulk nuclear DNA in both immature and mature erythrocytes. This study confirms and extends the results of Weintraub and Groudine (16) and Garel et al. (20) and reveals that the relative rate of digestion of globin genes in erythroid cells is independent of the rate of globin mRNA transcription.

The globin genes are selectively destroyed by the extensive DNase I digestion of mature erythrocyte nuclei (16) (Fig. 6), whereas these same sequences are not preferentially cleaved by mild digestion of these nuclei with micrococcal nuclease (Figs. 4 and 5). To determine if this difference resulted from differences in the types of analysis employed in these studies, we have examined the effects of a mild DNase I digestion of erythroid nuclei on the selective cleavage of the globin genes. Nuclei were incubated with DNase I (10 μg/ml) at 0°C for 4 min, at which time 1 to 3% of the DNA was rendered acid-soluble. Less than 5% of the globin genes in immature or mature erythrocytes were destroyed by this mild digestion (Fig. 6). The DNA extracted from these nuclei was separated into four molecular weight classes on native agarose gels as described in Fig. 1 and under “Experimental Procedures.”

Analysis of these samples on native polyacrylamide gels (Fig. 7, native) revealed that the native DNA lengths in each size class were similar to the corresponding DNA lengths obtained from micrococcal nuclease digested nuclei (Fig. 1). On denaturing gels (Fig. 7, denatured), size classes 1 through 4 contained single-stranded DNA of increasing molecular weight averages, although the 10 base periodicity, characteristic of DNA from DNase I digested nuclei (37), was present in the DNA from each size class. Hybridization of globin cDNA in the four size classes revealed an inverse relationship between the concentration of globin genes and the length of the DNA in both immature and mature erythrocytes (Figs. 8, A and B). The concentration of globin sequences in the smallest size class (less than 300 base pairs) from immature erythrocyte nuclei was 7-fold greater than the concentration of these sequences in undigested DNA and 17-fold greater than in the fraction containing DNA duplexes >1200 base pairs. Similarly, the concentration of globin coding DNA in the smallest size class isolated from mature erythrocyte nuclei was 6-fold more than in undigested DNA and 12-fold greater than in the largest DNA size class. The selective cleavage of globin genes
Conformational Changes of Genes in Chromatin

FIG. 8. Distribution of ovalbumin and globin genes in different size DNA fragments. Nuclei were digested with DNase I (10 μg/ml) for 4 min at 0°C. DNA was extracted from the digested nuclei and separated into four molecular weight classes as described in Fig. 1. The two larger molecular weight classes were sonicated as described under "Experimental Procedures." DNA (0.375 mg/ml) from each size class was annealed to 3.4 x 10⁴ cpm/ml of ovalbumin or globin cDNA for the times indicated. A, nuclei from immature erythrocytes were digested with DNase I as described above and the resulting DNA was annealed with globin cDNA. The size classes 1 (M), 2 (X-X), 3 (X-X), and 4 (M) were similar to the fragments shown in Lanes 1, 2, 3, and 4 of Fig. 1 and contained 5, 21, 27, and 45% of the DNA, respectively. B, DNA from mature erythrocyte nuclei was prepared as described above and annealed with globin cDNA. The symbols are the same as in A and the percentages of DNA in size classes 1 to 4 were 3, 7, 19, and 71, respectively. C, DNA from adult hen oviduct nuclei was annealed with ovalbumin cDNA. The symbols are the same as in A and the percentages of DNA in size classes 1 to 4 were 4, 8, 17, and 71, respectively. D, the same DNA samples for hybridization as in C were annealed with globin cDNA. Symbols designating the DNA size classes are the same as in A.

DISCUSSION

The tissue and developmental stage specific expression of ovalbumin and globin genes is regulated, in part, at the level of transcription (22, 23, 25, 31, 32). Although there is no cytological or electron microscopic evidence relating the structure of the ovalbumin and globin genes in chromatin to their transcriptional activities, such studies derived from a variety of other eukaryotic systems have revealed a close correlation between changing patterns of gene expression and dynamic alterations in the chromatin conformation of these gene sequences (see the introduction to the text for references). Previous studies have shown that transcribing ovalbumin genes in hen oviducts (21, 22), genes coding for total poly(A) mRNA in trout testes (38), and transcriptionally active ribosomal RNA genes in amphibian oocytes (39,40) are packaged in structural configurations which are preferentially recognized by micrococcal nuclease. The results presented in this investigation suggest that the association of this micrococcal nuclease-sensitive structure with the ovalbumin and globin genes in chromatin is a dynamic process which appears to be correlated with alterations in the expression of these specific sequences. The decrease in the transcription of the ovalbumin gene in the chick oviduct following estrogen withdrawal (23), and the transcriptional inactivation of the globin coding DNA during the final stages or erythropoiesis (25) are both accompanied by a reduction in the ability of micrococcal nuclease to recognize selectively and to excise these specific sequences from chromatin (Figs. 1 to 5). Additional support for the dynamic relationship between transcription and the chroma-
tin structure recognized by this nuclease was provided by the studies of Reeves (39, 40) which revealed striking correlations between the rates of ribosomal RNA synthesis and the sensitivity of the ribosomal coding DNA to micrococcal nuclease during oogenesis and embryogenesis in Xenopus laevis. Whether micrococcal nuclease recognizes a property of chromatin which is responsible for selective gene transcription or whether this enzyme attacks a chromatin component which is a consequence of the transcriptional process is a problem for future investigation.

Several structural and biochemical features are characteristic of the transcriptionally active regions of chromatin (9, 41–43). Enzymatic probes for these regions, therefore, may recognize different properties associated with expressed genes. Transcribing gene sequences are recognized preferentially by both micrococcal nuclease and DNase I. The properties of these sequences in chromatin which are recognized by micrococcal nuclease sensitivity are apparently lost during transcriptional inactivation (Figs. 1 to 5). As first shown by Weintraub and Groudine (16) and later by other investigators (17, 44) (Fig. 6), however, the globin genes retain their sensitivity to DNase I digestion in erythroid cells which are no longer synthesizing globin mRNA. Fig. 8 also demonstrates that DNase I cleaves preferentially transcribing genes early in the digestion, but, in contrast to micrococcal nuclease, DNase I also cleaves selectively the transcriptionally inactive globin genes in the mature erythrocyte. In addition, when 80 to 90% of the estrogen receptors have been lost from oviduct nuclei after estrogen withdrawal, the ovalbumin genes retain their selective sensitivity to DNase I digestion (19). Inhibition of ovalbumin mRNA accumulation with the anti-estrogen tamoxifen is also without effect on the DNase I sensitivity of the ovalbumin genes in the chick oviduct (18). These observations are consistent with the proposal (16) that the property of chromatin recognized selectively by DNase I is imposed on specific genes as they are transcriptionally activated and remain associated with these sequences following their inactivation. The maintenance of an altered conformation about inactivated genes may play an important role in the selective reactivation of the globin genes in transformed erythroleukemic cells (45–47), previously extinguished genes in cell hybrids (48), and fetal specific genes in adult neoplastic cells (49).

The molecular basis for the enhanced sensitivity of specific genes in chromatin to nuclease probes is poorly understood. The results of this study suggest that DNase I and micrococcal nuclease may recognize different features associated with the globin genes in hen erythroid chromatin since these sequences in the mature erythrocyte remain selectively sensitive to DNase I but not to micrococcal nuclease (Figs. 4 to 8). DNase I attacks the DNA associated with nucleosome core histones even during a brief digestion (37). The specificity of this enzyme for active or formerly active genes may be related to altered nucleosomal conformations along these sequences or to higher orders of chromatin folding which are generated by these altered subunits (16, 50–52). The liberation of small DNA fragments enriched in globin genes during the mild digestion of erythroid nuclei with DNase I (Fig. 8), therefore, may result from the selective intranucleosomal cleavage of these sequences or from cleavage of adjacent nucleosomes associated with the globin genes. During mild digestions of nuclei with micrococcal nuclease, such as those employed in this study, cleavage is restricted to the DNA fiber connecting adjacent nucleosomes (53, 54). Whether the specificity of micrococcal nuclease for actively transcribing genes in chromatin results from reduced nucleosome protection of linker DNA in the active regions, altered higher order structure of the transcribed chromatin fiber, or some other property of the active chromatin complex is not known at the present time. Further characterization of the chromatin conformations recognized selectively by micrococcal nuclease and DNase I, and elucidation of the mechanisms by which these conformations are formed, should lead to a better understanding of the complex relationships between chromatin structure and specific gene activity.

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