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Selective digestion of transcriptionally active ovalbumin genes from oviduct nuclei

(deoxyribonuclease I digestion/transcription/DNA reassociation)

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ABSTRACT **Analysis of the DNA of isolated nucleosomes suggests that virtually all genomic DNA sequences are organized in this basic chromatin subunit. In this report, we demonstrate that although histones reside on the transcriptionally active ovalbumin genes in the oviduct, the organization of proteins about this gene renders it highly sensitive to deoxyribonuclease I (deoxyribonucleate 5′-oligonucleotidohydrolase, EC 3.1.4.5). Treatment of oviduct nuclei from the laying hen with pancreatic deoxyribonuclease I results in the preferential digestion of over 70% of the ovalbumin sequences when only 10% of the total nuclear DNA has been solubilized. Treatment of liver nuclei does not reveal selective sensitivity of these genes to DNase I. Furthermore, regions of DNA not actively transcribed, such as the endogenous leukosis virus genes in the oviduct, are not selectively degraded by this enzyme. Similar digestions with micrococcal nuclease, however, reveal no specific digestion of transcriptionally active chromatin. These data confirm the observations of H. Weintraub and M. Groudine [(1976) *Science* 193, 848–856] and suggest we are dealing with an aspect of structure that may be necessary to permit transcription of the chromatin complex.**

The genome of all eukaryotes is associated with a stable complement of histone protein and a variable amount of nonhistone protein to form a complex that we define as chromatin. Control of gene expression is likely to result, at least in part, from the specific interaction of these proteins with DNA, in such a way as to permit the transcription of a given set of genes in one tissue while restricting their expression in other tissues. It is therefore likely that structural changes occur within the chromatin complex, which may be responsible for the induction and maintenance of the transcription of specific genes.

One basic structural component of the chromosome results from the organization of histone and DNA into a regular array of nucleosomes (1–7). This basic unit of structure involves about 190 base pairs of DNA complexed with two each of the four major histones and encompasses virtually all genomic sequences. Analysis of the distribution of nucleosomes over transcribing sequences reveals that the template-active regions of the genome are also organized in this basic structure (8–10). The mere presence of nucleosomes along a given DNA sequence is therefore not sufficient to prohibit transcription.

Considerable evidence exists to suggest that the template-active regions of chromatin are structurally distinct from nontranscribing segments of the genome. These aspects of structure are therefore not apparent in an analysis of nucleosome distribution which utilizes micrococcal nuclease. Bacterial RNA polymerase is capable of tissue-specific transcription from chromatin templates, a phenomenon likely to result from structural differences along active genes rather than from recognition of eukaryotic promoters (11–15). In addition, investigators have exploited the enhanced susceptibility of transcribing genes to deoxyribonucleases to isolate template-active from inactive chromatin (16, 17). More recently, Weintraub and Groudine (18) have demonstrated that the globin genes in avian erythrocytes are selectively sensitive to digestion with pancreatic deoxyribonuclease I (DNase I, deoxyribonucleate 5′-oligonucleotidohydrolase, EC 3.1.4.5). This important study has provided a consistent structural parameter distinguishing active from inactive regions. of chromatin.

In this report, we examine the susceptibility of the ovalbumin gene to cleavage by DNase I and micrococcal nuclease (nucleate 3′-oligonucleotidohydrolase, EC 3.1.4.7). In accord with the data of Weintraub and Groudine (18), we find that the ovalbumin genes are selectively digested by DNase I only in oviduct cells actively expressing these sequences. Selective cleavage of the ovalbumin genes is not observed with micrococcal nuclease. The sensitivity of the active genes is lost when isolated nucleosomes, prepared with micrococcal nuclease, are used as a substrate for DNase I.

MATERIALS AND METHODS

Deoxyribonuclease Digestion of Nuclei and Isolated Nucleosomes. Nuclei were obtained from the mucosa of the magnum portion of the oviduct of laying hens by homogenization and washings with sucrose-Triton X-100 buffers (11). For pancreatic DNase I digestions (Worthington Biochemical Corp.), nuclei were washed in 0.01 M Tris·HCl, at pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$ and resuspended in the same buffer at a DNA concentration of 1 mg/ml. Reactions were carried out at a DNase I concentration of 20 μg/ml at 37°, and the kinetics of digestion were assayed by measuring the amount of A_{260}-absorbing material that was soluble in 1 M $HClO_4$–1 M NaCl. Resistant DNA was purified free of protein and RNA, as described previously (19).

Purified nucleosomes were prepared from intact nuclei by initial digestion with micrococcal nuclease in 0.01 Tris·HCl at pH 7.9, 0.1 mM $CaCl_2$, 3% sucrose (wt/vol). Nuclei were resuspended at a concentration of 1 mg/ml and digested with micrococcal nuclease (1.5 μg/ml) at 37° to yield about 10% digestion of DNA. The reaction was terminated by the addition of Na EDTA to 5 mM. Nucleosomes were isolated from this reaction by sucrose gradient velocity sedimentation as previously described (8).

DNase I digestions of purified nucleosomes were performed

Abbreviations: C_0t, initial concentration of total DNA (moles of nucleotide/liter) × time (seconds); $C_0t_{1/2}$, ½ value of initial concentration of total DNA (moles of nucleotide/liter) × time; R_0t, initial concentration of total RNA (moles of nucleotide/liter) ×. time (seconds); $R_0t_{1/2}$, ½ value of initial concentration of total RNA (moles of nucleotide/liter) × time (seconds).

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by dialyzing the subunits against DNase I buffer. Digestion was carried out as described above until 15% of the nucleosomal DNA was solubilized. In a second set of experiments, DNase I digestion was carried out on micrococcal nuclease-treated nuclei without gradient purification of individual subunits. Nuclei were digested with micrococcal nuclease in 0.01 M Tris·HCl, at pH 7.9, 10 mM NaCl, 3 mM $MgCl_2$, 0.1 mM $CaCl_2$. After 10% of the DNA was solubilized, the nuclei were sedimented, resuspended in DNase I buffer, and digested further until an additional 15% of the DNA was solubilized with DNase I.

Gel Electrophoresis. Analysis of the single-stranded DNA product of digestion of nuclei and nucleosomes was performed by electrophoresis on 10% polyacrylamide/7 M urea gels as described by Maniatis *et al.* (20). Twenty-five micrograms of DNA was denatured by boiling for 3 min in 90% formamide. The samples were immediately cooled on ice, loaded on 17 cm slab gels, and electrophoresed at 200 V at room temperature. Gels were stained for 2 hr in ethidium bromide (1 μg/ml) and photographed under UV illumination.

Synthesis of cDNA. Ovalbumin mRNA was purfied from chicken oviducts by immune precipitation of ovalbumin synthesizing polyribosomes (G. Schutz, S. Kieval, B. Groner, D. Kurtz, and P. Feigelson, submitted for publication). Avian myeloblastosis 70S RNA was purified from intact virions. [^{3}H]DNA copies complementary to the above RNA preparations were prepared in 100 μl of the following reaction mixture: 0.05 M Tris·HCl at pH 7.9, 80 mM NaCl, 8 mM $MgCl_2$, 5 mM dithiothreitol, 0.2 mM each of dGTP and dATP, 0.1 mM [^{3}H]dCTP (23 Ci/mM), 0.1 mM dTTP (46 Ci/mM), 100 μg/ml of actinomycin D, 100 μg/ml of distamycin, 30 μg of poly(A)-containing RNA, and 3 μg of oligo(dT)-12–18. After 30 min at 37°, the reaction was terminated by the addition of sodium dodecyl sulfate to 0.2% and 20 μg of *Escherichia coli* carrier DNA was added. The [^{3}H]DNA product was purified free of protein, template RNA, and triphosphate by phenol extraction and G-50 chromatography as described previously (11). The specific activity of this cDNA was estimated to be 5×10^7 cpm/μg.

Annealing Reactions. Annealing reactions, with cDNA probes and excess quantities of nuclear DNA were performed in 1 mM Tris·HCl at pH 7.9, 0.2 mM EDTA, 0.4 M NaCl at 69° under paraffin oil. Duplex formation was assayed at various times in the reaction by treatment with S_1 nuclease (Miles Laboratories). Aliquots (10 μl) were removed and diluted in 1 ml of 0.03 M sodium acetate at pH 4.5, 0.1 M NaCl, 0.12 mM $ZnSO_4$. An aliquot (0.5 ml) was immediately precipitated with 10% trichloroacetic acid, while the other half was digested with S_1 nuclease (1000 units) for 1 hr at 37° prior to trichloroacetic acid precipitation.

RESULTS

DNase I digestion of ovalbumin genes in nuclei

Each tubular gland cell of the oviduct of the laying hen contains about 100,000 ovalbumin mRNA molecules which are responsible for over half the total protein synthesis of the cell (21). This response to complex hormonal stimuli is likely to result, in part, from adaptive changes regulating the transcription of the ovalbumin gene. We have therefore examined the accessibility of the ovalbumin gene to DNase I digestion in intact oviduct and liver nuclei. Digestion of oviduct nuclei with DNase I results in the generation of a set of DNA products, which upon denaturation reveal discrete single-stranded fragments at 10 nucleotide intervals from 20 to 160 base pairs (22, 23). The DNA

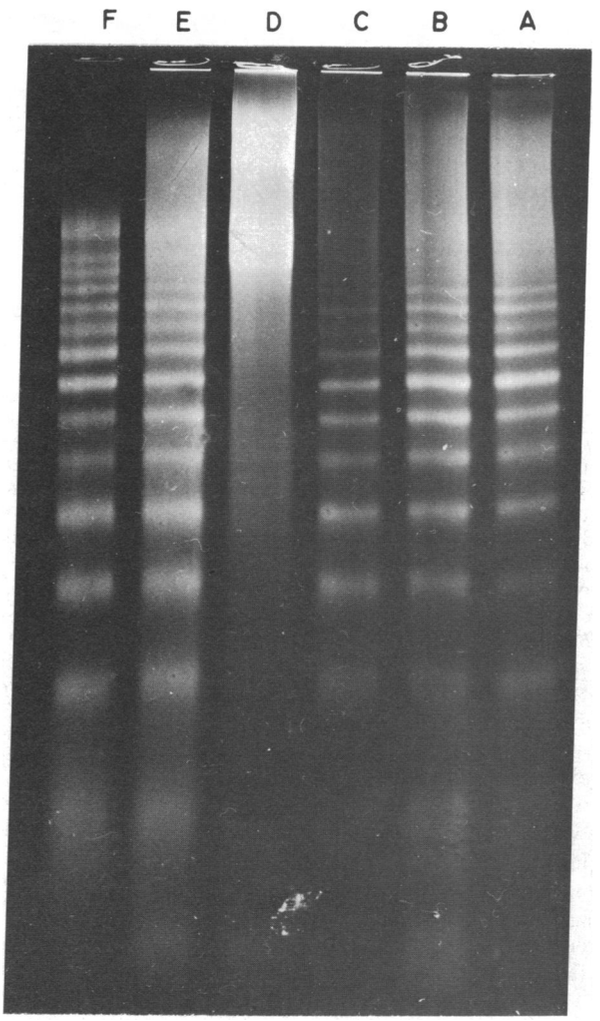

FIG. 1. Ten percent polyacrylamide/7 M urea gel electrophoresis of nuclear digestion products. Nuclei were digested as described below and 25 μg of the resistant DNA was denatured and applied to each slot. (A and B) 5 and 11% digestion of oviduct nuclear DNA in intact nuclei with DNase I, respectively; (C) 15% digest of liver nuclear; (D) 15% micrococcal nuclease digest of oviduct nuclei; (E) 15% micrococcal nuclease digestion of nuclei followed by 15% digestion with DNase I; (F) 15% DNase I digestion of isolated nucleosomes.

products of a 5 and 11% digestion of oviduct and liver nuclei are shown on a 7 M urea/10% polyacrylamide gel (Fig. 1, slots A, B, and C). This array of fragments appears very early in the digestion process, with the generation of these fragments that proceeds at the expense of the poorly resolved higher molecular weight DNA. It is of interest that with DNase I, even when 5% of the nuclear DNA significant cleavage of intranucleosomal DNA has occurred. This finding indicates that the DNA within the nucleosome may be as accessible to cleavage by DNase I as the DNA bridging the individual nucleosomal subunits.

We then examined the sensitivity of the ovalbumin genes to DNase I digestion in liver and oviduct nuclei in annealing reactions with ovalbumin cDNA. Ovalbumin mRNA was purified by immune precipitation of ovalbumin-synthesizing polyribosomes and a complementary cDNA copy synthesized with reverse transcriptase. Ninety percent of the cDNA prepared in this way anneals to ovalbumin mRNA which displays a single first-order transition with an $R_0t_{1/2}$ of 8×10^{-4}. No annealing is observed with total liver RNA at R_0t values of 10,000 which indicates that 90% of our cDNA is complementary to ovalbumin

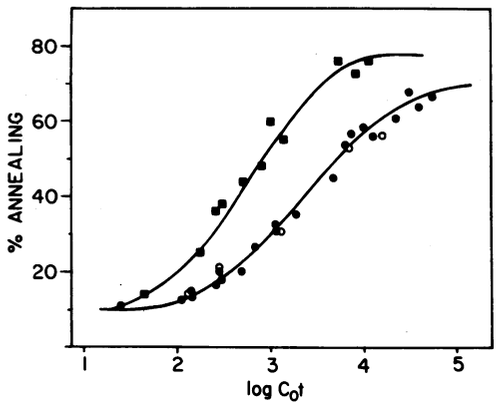

FIG. 2. Kinetics of annealing of ovalbumin cDNA with DNase I-treated nuclear DNA. Oviduct (●) and liver (■) nuclei were digested with DNase I until 15% of the DNA was acid soluble. The resistant DNA was purified free of protein and annealed with ovalbumin cDNA. In a separate experiment, oviduct nuclei were pretreated with RNase A and T_1 prior to DNase I digestion (○). Annealing reactions (200 μl) contained 4 mg of oviduct or liver DNA and 0.18 μg of ovalbumin cDNA. Duplex formation was monitored with S_1 nuclease.

mRNA. R_0t refers to initial concentration of total RNA (moles of nucleotide/liter) × time (seconds). $R_0t_{1/2}$ refers to ½ value of initial concentration of total RNA (moles of nucleotide/liter) × time (seconds).

Fig. 2 presents the kinetics of annealing of this cDNA to a large excess of liver and oviduct DNA following DNase I treatment. The annealing of cDNA to digested liver DNA occurs with $C_0t_{1/2}$ of 600 with over 80% of the cDNA-forming duplex at saturation. The kinetics of annealing of cDNA with digested oviduct DNA reveals a second-order transition with $C_0t_{1/2}$ of 2000, a value 3.3 times that observed either with digested liver DNA or with total oviduct DNA. This reaction approaches a saturation plateau at a C_0t of 50,000 and total DNA/cDNA ratios of 2×10^7 are required to reach these levels of annealing. The ovalbumin genes are therefore specifically sensitive to the DNase I digestion only in those tissues actively engaged in ovalbumin RNA synthesis.

The annealing data are displayed in a semilogarithmic plot in Fig. 2. In Fig. 3, the second-order rate equation for the reassociation of DNA (24) is transformed to $H/1 - H = D_0kt$, where H is the extent of annealing at time t, D_0 represents the initial concentration of ovalbumin genes, and k is the rate of reassociation of ovalbumin DNA. The slope of the line generated by plotting $H/1 - H$ as a function of t is proportional to the ovalbumin gene content. This calculation assumes that the sizes of the annealing species be constant in all reactions. From Fig. 3, we clearly observe that the kinetics of annealing of ovalbumin cDNA to DNA obtained from DNase-treated oviduct nuclear is 3-fold slower than that observed with similar preparations of liver DNA.

From these data, it is apparent that although there is selective digestion of the transcriptionally active ovalbumin genes, the resultant oviduct DNA is still capable of annealing to ovalbumin cDNA at ⅓ the rate of similarly treated liver nuclear DNA. It was therefore of interest to determine if more extensive digestion with DNase I would ultimately result in specific digestion of these resistant sequences. Oviduct nuclei were digested to 20 and 26% solubilization of DNA. Annealing of these DNA products with ovalbumin cDNA again reveals a $C_0t_{1/2}$ of 2000. This resistant DNA does not represent a restricted region of the ovalbumin gene, because annealing to this DNA saturates the cDNA probe at a value identical to that observed with total DNA, albeit at a higher C_0t value.

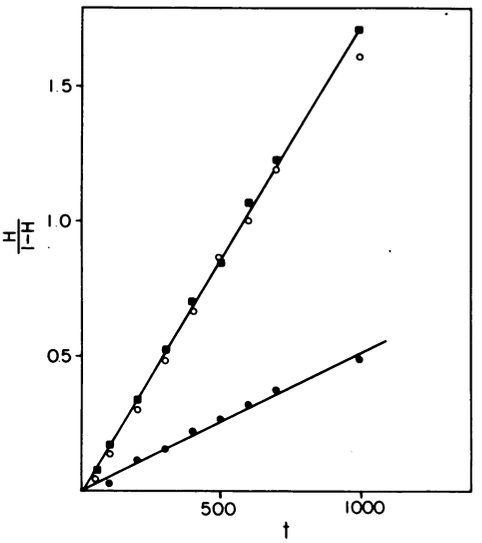

FIG. 3. Linear representation of the kinetics of annealing of ovalbumin cDNA to DNase I-treated nuclear DNA. The data in Figs. 2 and 5 are plotted in a linear fashion. As described in the legends to these figures, ovalbumin cDNA was annealed with DNA derived from DNase I-treated oviduct nuclei (●), DNase I-treated liver nuclei (○), or DNase I-treated oviduct nucleosomes (■).

DNase sensitivity of unexpressed genes

These studies confirm the observations of Weintraub and Groudine for the globin genes and reveal that the template-active segments are organized in chromatin in such a way as to render them exceedingly sensitive to DNase I attack. We would predict, then, that genes inactive in both liver and oviduct should be present in DNase-treated preparations at concentrations identical to that of undigested total DNA. One such genome amenable to this analysis is the sequence of the avian myeloblastosis virus (AMV). Sixty percent of the viral genome is integrated in uninfected chick cells (25), but these sequences are not normally expressed in adult liver or oviduct. We therefore annealed avian myeloblastosis virus cDNA to DNase-treated liver and oviduct DNAs used in Fig. 2. We find that there is no selective digestion of these sequences in nuclei derived from either tissue (Fig. 4). The $C_0t_{1/2}$ value of 600 is twice that observed with total sonicated DNA, a finding consistent with the differences in the lengths of the DNA populations. These data support our observations with the ovalbumin genes and indicate that selective sensitivity of the specific se-

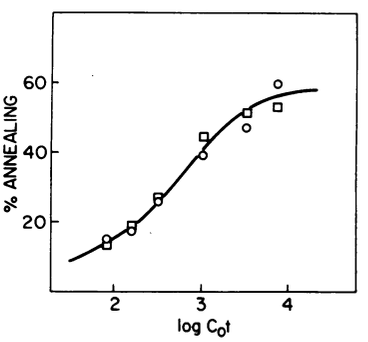

FIG. 4. Kinetics of annealing of avian myeloblastosis virus cDNA with DNase I-treated nuclear DNA. Oviduct and liver nuclei were digested with DNase I and the resistant DNA was purified free of protein. The resistant DNA (2.5 mg) from oviduct (○) and liver (□) nuclei was annealed to 0.18 ng of viral cDNA under conditions described in Fig. 2.

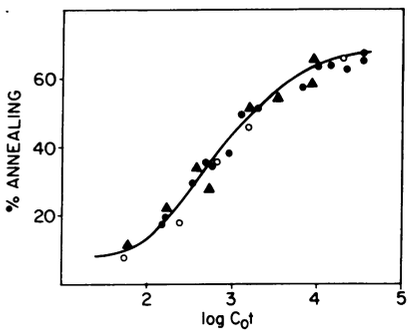

FIG. 5. Kinetics of annealing of ovalbumin cDNA with DNA that results from DNase I digestion of isolated nucleosomes. Nucleosomes were isolated from oviduct nuclei by micrococcal nuclease digestion, and then by velocity centrifugation. The isolated nucleosomes were then digested with DNase I until 15% of the DNA was solubilized. DNA was purified from nucleosomes prior to (●) and after DNase I digestion (▲), and was annealed with ovalbumin cDNA as described in the legend to Fig. 2. In a separate experiment, nuclei were digested with micrococcal nuclease until 15% of the DNA was solubilized, centrifuged, and subjected to a second digestion with DNase I until a further 15% of the DNA was solubilized. The resistant DNA was then annealed with ovalbumin cDNA (○).

quences to DNase I may reflect a structural parameter directly related to transcriptional activity.

DNase I digestion of nucleosomes

Micrococcal nuclease cleavage of intact nuclei results in the generation of multimeric and monomeric nucleosomal DNA. Unlike DNase I, at early times in the digestion process, virtually no digestion occurs within the nucleosomal subunit with cleavage restricted to the DNA bridging individual subunits. In previous studies, we examined the distribution of nucleosomes along transcribing genes by annealing globin cDNAs to nucleosomal DNA from erythroblasts actively engaged in globin RNA synthesis. These experiments (8), along with more recent studies (9, 10), indicate that nucleosomes exist on transcriptionally active regions of the chromosomes.

The enhanced susceptibility of the ovalbumin genes to DNase attack prompted us to perform similar experiments on the distribution of nucleosomes about these genes in oviduct nuclei. Thus, nuclei were digested with micrococcal nuclease until 15% of the DNA was solubilized. Digestion to this level results in the liberation of over 80% of the DNA in the form of monomeric particles. DNA was then extracted directly from this digest of nuclei. In a separate experiment, nuclei were digested with micrococcal nuclease, and monomeric nucleoprotein subunits were isolated by velocity centrifugation. DNA was then purified from these preparations of isolated monomeric particles. The kinetics of annealing of these two DNA preparations with ovalbumin cDNA are shown in Fig. 5. The $C_0t_{1/2}$ of these reactions is 650, a value similar to that observed with DNse I-treated liver nuclei. These data are in accord with similar studies in other systems and indicate that nucleosomes reside along the ovalbumin genes even in a tissue actively synthesizing ovalbumin RNA. Micrococcal nuclease, unlike DNase I, does not selectively cleave the ovalbumin genes in oviduct nuclei. DNase I is therefore capable of recognizing an aspect of structure about the ovalbumin genes not recognized by micrococcal nuclease.

It was now of obvious interest to determine whether the structural property that renders the ovalbumin gene specifically sensitive to DNase is retained in isolated nucleosomes. The experimental approach we have chosen simply involves the isolation of nucleosomes by micrococcal nuclease digestion followed by DNase I digestion. The concentration of ovalbumin genes in the resistant DNA is then determined in annealing reactions. Nuclei were digested with micrococcal nuclease in buffers identical to those used for DNase I digestions. After 15% of the DNA was solubilized, the nuclei were spun out of the micrococcal nuclease buffer, resuspended, and digested an additional 15% with DNase I. In a separate experiment, individual nucleosomes were purified by sucrose gradient centrifugation and then further digested with DNase I. It should be noted that although the nucleosomes precipitate under the conditions of DNase I digestion, the pattern of repeated DNA fragments at 10 base pair intervals is virtually identical to that obtained by direct DNase I treatment of intact nuclei (Fig. 1).

The annealing profiles of these DNA preparations with ovalbumin cDNA are shown in Fig. 5. The kinetics of annealing of DNA obtained from DNase-treated nucleosomes are identical to those of untreated preparations of nucleosomal DNA. The observed $C_0t_{1/2}$ of 650 is identical to that of untreated nucleosomal DNA. These data are also displayed on a linear plot in Fig. 3. Active genes in isolated nucleosomes no longer appear to retain the "active conformation" that renders these genes highly sensitive to DNase I in intact nuclei. These studies implicate long-range effects in maintaining the conformation about active genes, because this conformation is apparently lost when genes are cleaved into individual nucleosomal subunits.

DISCUSSION

The elucidation of a regular array of nucleosomal subunits in chromatin immediately poses the question as to the possible role of this structure in the process of transcription. Analysis of the organization of this subunit structure with respect to DNA sequence reveals that no long special sequence of nucleotides is necessary for nucleosome formation. Virtually all genomic sequences are involved in the repeat structure which suggests a random distribution of nucleosomes along the genomic DNA (8). In this report, we demonstrate that nucleosomes are present along the ovalbumin genes of the oviduct, a finding consistent with previous studies indicating the presence of histone along actively transcribing regions of the genome.

We would predict, however, that the organzation of proteins about template-active genes should be distinct from that observed on unexpressed segments of the genome. Convincing support for this idea is provided by the recent experiments of Weintraub and Groudine (18), which demonstrate the selective digestion of the globin genes by DNase I in erythroid tissues. Our data on the structure of the ovalbumin genes in chromatin support the belief that the transcribing genes are maintained in an "active conformation" that renders them exceedingly sensitive to attack by DNase I. When 10% of the total DNA is solubilized over 70% of the ovalbumin genes are specifically digested in oviduct nuclei. The selective digestion is not observed in liver nuclei. Furthermore, the endogenous viral genes, which are not actively transcribed in the oviduct are not selectively degraded by this enzyme.

The observation that residual annealing at a reduced rate occurs between ovalbumin cDNA and DNA derived from extensively digested oviduct nuclei warrants further comment. Although it is apparent that there is selective cleavage of the active genes in chromatin, we do not at present know the nature of the digestion products. At one extreme, it is possible that the DNA in this active configuration is digested either to acid-soluble oligonucleotides or to small fragments incapable of forming stable duplexes under our annealing conditions. The

resistant ovalbumin sequences observed after DNase I treatment would then be attributable to subsets of ovalbumin genes that behave in these experiments as if they were transcriptionally inactive. These observations would then be in accord with the histologic findings that demonstrate that only 75–80% of the oviduct cells are engaged in ovalbumin mRNA synthesis. Alternatively, the DNA of active genes could be cleaved to small fragments that participate in annealing reactions at rates far below that observed with inactive gene fragments. If cleavage within active genes results in the liberation of discrete low molecular weight DNA fragments, then the size distribution of this set of DNA may ultimately reflect the internal structure of the nucleosome about transcribing genes.

Several interesting questions emerge as a result of these studies. Current data provide no information that identifies those factors responsible for the induction and maintenance of the conformation of active genes. Perhaps the simplest explanation for the enhanced DNase sensitivity is that it results from the presence of some component of the transcriptional complex that involves polymerase or ribonucleoprotein. In preliminary experiments, we have investigated this possibility by treatment of oviduct nuclei with ribonucleases prior to DNase I digestion (Fig. 2). Pretreatment of nuclei with ribonuclease has no effect upon the sensitivity of the ovalbumin genes to DNase I action. We have no evidence, however, on the effectiveness of this treatment in destroying nascent RNA bound to its chromatin template. In addition, it has been shown (18), that the adult globin genes remain in an active conformation in mature erythocytes, even though globin RNA synthesis has ceased. We therefore think it unlikely that the presence of the transcriptional complex upon a given region is responsible for the observed conformational differences.

A second problem involves the generation of this active structure through an entire gene consisting of several nucleosomes. Does the formation of such a structure involve multiple local events resulting in the modification of each nucleosome or is a distant structural effect transmitted through the nucleosomes of a given gene? It is possible that the addition of a specific nonhistone protein or the modification of histones within the nucleosomes distributed about a given region of chromatin results in the generation of a structure distinguishable by its sensitivity to DNase I action. Alternatively, this structure may reflect disruption in the higher order folding of the chain of nucleosomes resulting in unfolding of the individual nucleosomal subunits, perhaps in a manner described by Alberts *et al.* (26, 27). Our data that examine the sensitivity of the ovalbumin sequences in isolated nucleosomes to DNase I action support the latter alternative. A note of caution should be added in the interpretation of these findings. Care has been taken to prepare nucleosomes in buffers that maintain this structure in intact nuclei. We cannot, however, rule out the possibility that the actual process of digestion with micrococcal nuclease may simply destroy this structure perhaps by dislodging an essential nonhistone protein. It is also possible that although local events modify nucleosomes along a given gene, the structure about these segments of chromatin is reversible and this reversal is effected by some aspect of the digestion process.

A final point involves the generality of the observation that active genes maintain a conformation exceedingly sensitive to DNase I attack. The initial studies of Weintraub and Groudine examined the structure of the globin genes as well as those sequences transcribing nuclear RNA (18). In this report, we extend the observations to the ovalbumin genes in oviduct nuclei. Preliminary studies from our laboratory also indicate that newly integrated C-type viral genomes are also in this conformation in cells actively synthesizing viral RNA. The generality of this observation suggests that we are dealing with an aspect of structure necessary to permit transcription of the chromatin complex.

We wish to thank Drs. Weintraub and Groudine for making their data available to us prior to publication. In addition, we thank Dr. Raymond Sweet for providing AMV cDNA, and Drs. Feigelson, Killewich, Schutz, and Ernest for providing ovalbumin mRNA and for familiarizing us with the ovalbumin system. The helpful criticism of Dr. Sol Spiegelman is greatly appreciated. This investigation was supported by Grant no. CA-16346, awarded by the National Cancer Institute, DHEW.

1. Hewish, D. R. & Burgoyne, L. . (1973) *Biochem. Biophys. Res. Commun.* **52,** 504–510.
2. Noll, M. (1974) *Nature* **251,** 249–251.
3. Sahasrabuddhe, C. G. & Van Holde, K. E. (1974) *J. Biol. Chem.* **249,** 152–156.
4. Axel, R. (1975) *Biochemistry* **14,** 2921–2925.
5. Sollner-Webb, B. &. Felsenfeld, G. (1975) *Biochemistry* **14,** 2916–2920.
6. Kornberg, R. & Thomas, J. O. (1974) *Science* **184,** 865–868.
7. Olins, A. L., Carlson, R. D. & Olins, D. E. (1975) *J. Cell. Biol.* **64,** 528–537.
8. Lacy, E. & Axel, R. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3978–3982.
9. Tien Kuo, M., Sahasrabuddhe, C. G. & Saunders, G. F. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1572–1575.
10. Axel, R., Cedar, H. & Felsenfeld, G. (1975) *Biochemistry* **14,** 2489–2495.
11. Axel, R., Cedar, H. & Felsenfeld, G. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2029–2032.
12. Gilmour, R. S. & Paul, J. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3440–3442.
13. Steggles, A., Wilson, G., Kantor, J., Picciano, D., Falvry, A. & Anderson, W. F. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1219–1222.
14. Jacquet, M., Groner, Y., Monroy, G. & Hurwitz, J. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3045–3049.
15. Harris, S. E., Schwartz, R. J., Ming-Jer, T. & O'Malley, B. W. (1976) *J. Biol. Chem.* **251,** 524–529.
16. Gottesfeld, J., Murphy, R. F. & Bonner, J. (1975) *Proc. Natl. Acad. Sci USA* **72,** 4404–4408.
17. Berkowitz, C. & Doty, P. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3328–3332.
18. Weintraub, H. & Groudine, M. (1976) *Science,* **193,** 848–856.
19. Axel, R., Melchior, W., Sollner-Webb, B. & Felsenfeld, G. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4101–4105.
20. Maniatis, T., Jeffrey, A. & van de Sande, H. (1975) *Biochemistry* **14,** 3787–3794.
21. Palmiter, R. D. (1975) *Cell* **4,** 189–197.
22. Noll, M. (1974) *Nucleic Acid Res.* **1,** 1573–1578.
23. Camerini-Otero, D., Sollner-Webb, B. & Felsenfeld, G. (1976) *Cell* **8,** 333–347.
24. Britten, R. J. & Kohne, D. E. (1968) *Science* **161,** 529–543.
25. Shoyab, M., Baluda, M. A. & Evans, R. (1974) *J. Virol.* **13,** 331–339.
26. Weintraub, H., Alberts, B. & Worcel, A. (1976) *Cell,* in press.
27. Alberts, B., Weintraub, H. & Worcel, A. (1976) *Proc. of the Inter. Symp. on the Eukaryotic Genome,* in press.