Proc. Natl. Acad. Sci. USA
Vol. 74, No. 11, pp. 4867–4871, November 1977
Biochemistry

Genes transcribed at diverse rates have a similar conformation in chromatin

(chromatin structure/transcription/DNA reassociation)

ANNIE GAREL*, MIRIAM ZOLAN, AND RICHARD AXEL

Institute of Cancer Research and Department of Pathology, College of Physicians and Surgeons, Columbia University, 701 West 168th Street, New York, New York 10032

Communicated by S. Spiegelman, August 19, 1977

ABSTRACT **We have analyzed the DNA generated upon treatment of oviduct nuclei with pancreatic DNase I (deoxyribonucleate 3′-oligonucleotidohydrolase; EC 3.1.4.6), with cDNA copies of specific mRNA sequences to study the structure and organization of transcriptionally active genes in chromatin. In this report we examine the kinetics of digestion of three classes of genes in the oviduct which are transcribed at significantly different rates. Our results indicate that the ovalbumin genes appear to be organized by chromatin proteins in such a way that they are rendered exceedingly sensitive to digestion by DNase I. This sensitivity is not observed in the liver, a tissue in which these genes are transcriptionally inert. Furthermore, the transcriptionally inactive globin genes in the oviduct are not selectively sensitive to nuclease attack and are digested 5 times more slowly in the ovalbumin genes in this tissue. In addition, we have examined the accessibility of a complex subset of genes that are rarely represented in the mRNA and are likely to be transcribed at a frequency orders of magnitude below that of the ovalbumin gene. Comparison of the accessibility of these sequences with that of the ovalbumin gene indicates that these two subsets of genes are recognized and cleaved by DNase I at similar rates. These results suggest that the maintenance of an active conformation about specific genes does not reflect the polymerase distribution about these genes. This active conformation is therefore not confined to sequences actively engaged in the transcription process and may reflect the structure about a subpopulation of the genome which represents the transcriptional potential of a given cell type.**

Digestion of the eukaryotic chromosome with deoxyribonucleases has revealed the presence of periodic cleavage sites in DNA that liberate the nucleosome or basic subunit of the chromosome. Analysis of the DNA cleavage products in molecular hybridization reactions with cDNA transcripts of specific genes has demonstrated that this periodicity is maintained over transcriptionally active segments of the genome (1–6). Since the interaction of histones with DNA is responsible for the generation of the nucleosomal repeat, these studies suggest that histones reside along active genes and that the mere presence of histone is not sufficient to restrict transcription.

Significant morphologic and biochemical evidence exists, however, to suggest that the proteins of chromatin maintain these genes in a discrete active conformation that is distinguishable from the majority of the transcriptionally inert DNA in the chromosome. Perhaps the most convincing morphologic evidence for the presence of an altered structure about active genes derives from electron microscopic observations which reveal sites of RNA synthesis within the chromosome in a more diffuse and extended conformation. A striking example of this phenomenon is reflected in the puffs observed in the polytene chromosomes of Diptera (7). Recently, DNA binding proteins have been used to probe the accessibility of specific genes in chromatin. The addition of bacterial RNA polymerase to eukaryotic chromatin templates results in tissue-specific transcription (8–12), a phenomenon likely to result from structural differences along active genes rather than specific recognition of eukaryotic promoters. More recent studies have demonstrated that transcriptionally active genes are extremely sensitive to attack by a variety of deoxyribonucleases (2–4, 13). This selective digestion may reflect an unfolding of nucleosomes along these genes, which results in a more extended conformation (14) that is more accessible to nuclease attack.

In the present study, we have analyzed the kinetics of digestion of three classes of genes in the oviduct that are transcribed at significantly different rates: (*i*) the ovalbumin genes, which are transcribed rapidly and are therefore densely packed with polymerase molecules; (*ii*) a complex subset of genes that are rarely represented in the mRNA population and are likely to be transcribed at a frequency orders of magnitude below that of the ovalbumin gene; and (*iii*) the globin genes, which are not transcribed in the oviduct. Our results indicate that transcriptionally active genes are exceedingly sensitive to cleavage by DNase I (deoxyribonucleate 3′-oligonucleotidohydrolase; EC 3.1.4.6), but that the kinetics of digestion of these active segments of the chromosome are independent of transcription rates. These results suggest that the presence of an active transcriptional complex along a given gene is not required to maintain an active, DNase-sensitive conformation about these genes.

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 (14). For pancreatic DNase I digestions (Worthington Biochemical Corp.), nuclei were washed in 10 mM Tris·HCl, 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 by proteinase K digestion, phenol/chloroform extractions, and alkali hydrolysis, as described (3).

Synthesis of cDNA. Ovalbumin mRNA was purified from chicken oviducts by immune precipitation of ovalbumin-syn-

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: R_0t and C_0t, initial concentration of total RNA and DNA, respectively, (moles of nucleotide/liter) × time (sec).

* Visiting scientist from the Institut de Biologie Moleculaire et Cellulaire, Lyon, France.

thesizing polyribosomes (15). Total oviduct poly(A)-mRNA was purified from polyribosomes by phenol/chloroform extraction and oligo(dT)-cellulose as described (16). Radioactive cDNA complementary to specific mRNA preparations was prepared in 100 μl of the following reaction mixture: 50 mM Tris·HCl (pH 7.9), 80 mM NaCl, 8 mM $MgCl_2$, 5 mM dithiothreitol, 0.2 mM each of dATP, dGTP, dTTP, and [^{3}H]dCTP or [^{32}P]dCTP, 10 μg of actinomycin D, 2–5 μg of mRNA, 0.5 μg of oligo-$(dT)_{12-18}$, and 10 units of avian myeloblastosis virus polymerase. After 60 min at 37° the reaction was terminated by dilution in 1 ml of 0.4 M NaCl/20 mM Na-EDTA, pH 7.0/0.2% sodium dodecyl sulfate, and 20 μg of *Escherichia coli* DNA was added as carrier. The cDNA product was purified free of protein, RNA, and triphosphate by phenol extraction and Sephadex G-50 chromatography as described. Globin [^{3}H]cDNA was kindly provided by Pat Williamson and Gary Felsenfeld.

Annealing Reactions. Annealing reactions, with cDNA probes and excess quantities of nuclear DNA were performed in 1 mM Tris·HCl, 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 30 mM sodium acetate, 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.

Purification of Scarce Sequence cDNA. Total poly(A)-containing mRNA was prepared from oviduct polysomes as described (16). cDNA complementary to oviduct poly(A)-mRNA was synthesized with reverse transcriptase (RNA-dependent DNA polymerase) in the reaction mixture described above. Of this total cDNA 0.1 μg (2×10^6 cpm) was annealed with 10 μg of oviduct mRNA to an R_0t value of 2. (R_0t is the initial concentration of total RNA in moles of nucleotide/liter multiplied by time in sec.) At this R_0t value only those cDNAs that are represented by the more abundant mRNA classes are capable of duplex formation. The cDNA in hybrid was fractionated from single-stranded cDNA by bringing the entire annealing mixture to 10 mM sodium phosphate (pH 6.5), and loading it on a 1 × 0.5 cm hydroxylapatite column at 65°. The column was eluted first with 0.15 M sodium phosphate (pH 6.5), to remove single-stranded cDNA. The double-stranded cDNA was then eluted with 0.5 M sodium phosphate. At this point in the annealing reaction, 65% of the cDNA was in duplex form. The single-stranded cDNA was treated with 0.4 M NaOH for 12 hr to remove residual mRNA, neutralized and desalted by Sephadex G-50 chromatography, and concentrated by ethanol precipitation. This preparation of cDNA was now reannealed with 40 μg of total oviduct mRNA to an R_0t value of 200. The entire reaction mixture was then diluted into S_1 nuclease buffer (see below) and treated with S_1 nuclease to obtain the population of cDNA capable of entering into duplex formation. This final preparation of cDNA was purified by phenol/chloroform extraction, NaOH digestion of RNA, and ethanol precipitation. This cDNA preparation anneals to total poly(A)-mRNA as a single transition with an $R_0t_{1/2}$ of 35, with 75% of the cDNA hybridized at saturation.

Reconstitution of Chromatin. Chromatin was reconstituted from purified preparations of oviduct nuclear DNA and bovine histones at a protein-to-DNA ratio of 1.1:1 by sequential dialysis in urea/salt as follows. The histone–DNA mixture at a DNA concentration of 1 mg/ml was dialyzed against 50 volumes of 2 mM dithiothreitol/5 mM Tris·HCl, pH 7.9/5 M urea/2 M NaCl for 12 hr. Subsequent dialyses were performed for 1.5 hr against 5 M urea/1.5 M NaCl, 5 M urea/1.0 M NaCl, 0.8 M NaCl, 0.6 M NaCl (repeated twice), and 0.01 M NaCl. All of the above dialysis buffers contained 2 mM dithiothreitol/5 mM Tris·HCl, pH 7.9. The resultant chromatin reconstitute was then dialyzed into DNase I buffer as described above. Reconstitution performed in this fashion results in the generation of a chromatin complex that reveals the characteristic nucleosome profile and the 10-base-pair repeat when digested with appropriate deoxyribonucleases.

RESULTS

Selective digestion of ovalbumin gene in chromatin

We have previously demonstrated that the ovalbumin gene is preferentially digested by DNase I only in nuclei derived from the oviduct that is actively expressing ovalbumin mRNA (3). At present we have no information identifying those factors responsible for the induction and maintenance of this conformation about transcribed segments of the chromosome. At one extreme we could postulate that some component of the transcriptional apparatus involving either RNA polymerase or ribonucleoprotein is responsible for the enhanced DNase sensitivity. This view predicts that selective DNase sensitivity should only be observed when a specific gene is actively engaged in the process of transcription. Alternatively, it is possible that a subset of genomic sequences within the chromosome is maintained in an active conformation independent of the presence of RNA polymerase or nascent RNA. We have approached this problem experimentally by comparing the accessibility of the ovalbumin gene and the genes coding for the scarce mRNAs in chromatin to attack by DNase I. The oviduct of the laying hen is particularly amenable to this sort of analysis since the complexity measurements of polyadenylylated mRNA from this tissue have revealed that about half of the steady-state level of mRNA consists of ovalbumin mRNA sequences. An additional 30% of the message population consists of about 14,000 different mRNA sequences (scarce mRNA), each present only five times per cell (16).

We first examined the accessibility of the ovalbumin gene to DNase I attack by exposing oviduct nuclei to DNase I for increasing amounts of time to generate 3, 11, and 24% solubilization of nuclear DNA. The ovalbumin gene content was then determined by annealing a radioactive cDNA copy of immunologically purified ovalbumin mRNA to the DNA products of nuclease digestion (3, 15). In Fig. 1 we observe a series of second-order kinetic curves which demonstrate that, as the digestion process proceeds, the rate of reassociation of ovalbumin cDNA to DNase I-treated nuclear DNA is strikingly decreased. At 25% digestion, the $C_0t_{1/2}$ of the reaction has shifted to 2400, a value 4.3 times greater than that observed with total undigested nuclear DNA (Fig. 1). (C_0t is the initial concentration of DNA in moles of nucleotide/liter multiplied by time in sec.) This reduction in the reassociation rate represents a limiting value. Further digestion with DNase I results in no further diminution in the rate of reassociation with ovalbumin cDNA. The kinetics of attack of the ovalbumin genes are represented in composite form in Fig. 2. The data indicate that when only 7% of the nuclear DNA has been rendered acid soluble, there has been a 50% reduction in the rate of reassociation of the ovalbumin genes. Further digestion to 24% acid solubilization results in greater than a 75% reduction in reassociation rate.

Accessibility of inactive genes

These experiments indicate that the ovalbumin gene is preferentially digested by DNase I in oviduct nuclei. In previous

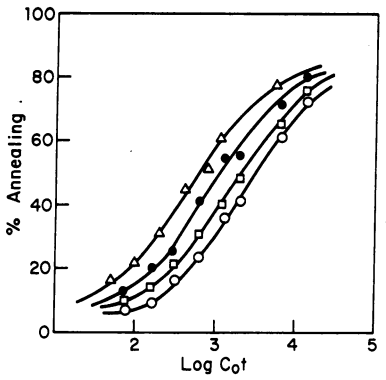

FIG. 1. Kinetics of annealing of ovalbumin cDNA with DNase I-treated oviduct nuclear DNA. Oviduct nuclei were digested for various times with DNase I and the resistant DNA was purified free of proteins and RNA. Ovalbumin [^{32}P]cDNA (0.12 ng) was incubated with 2.4 mg of sonicated total oviduct DNA (△), and DNA extracted from DNase I-treated nuclei after 3% (●), 11% (□), and 24% (○) solubilization. Annealing reactions were performed at 69° and duplex formation was monitored with S_1 nuclease.

studies we demonstrated that this preferential digestion was not observed with liver nuclei (3), suggesting that selectivity to the digestion process was related in some way to the transcription of the ovalbumin gene. Further support for this correlation between DNase I sensitivity and transcription would be obtained if we could demonstrate that transcriptionally inactive genes within oviduct nuclei are not preferentially digested by DNase I. To this end we examined the kinetics of digestion of the globin genes in oviduct nuclei in a manner analogous to that described for the ovalbumin genes. Experiments were performed in which ovalbumin [^{32}P]cDNA and globin [^{3}H]cDNA were annealed in the presence of a vast excess of DNase I-treated oviduct nuclear DNA. The kinetics of reassociation with the ovalbumin probe have already been presented in Fig. 1. In Fig. 3 we observe that DNase I treatment results in virtually no selective degradation of the globin genes in the oviduct. At 7% solubilization of nuclear DNA the rate of reassociation of globin cDNA is identical to that observed with sonicated, undigested nuclear DNA. Solubilization of DNA of 24% results in a 20% reduction in the annealing rate of globin cDNA, while the annealing with ovalbumin cDNA is reduced by a factor of 4.3. These experiments further support the conclusion that the transcriptionally active segments of the chromosome are or-

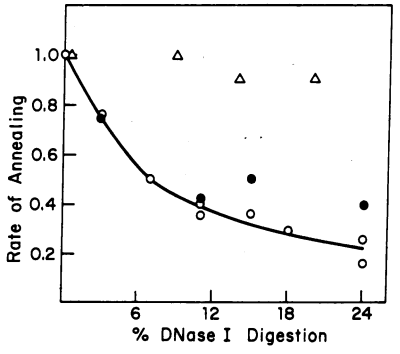

FIG. 2. Comparison of the rate of digestion of ovalbumin, globin, and rare sequence genes after DNase I digestion of oviduct nuclei. Composite of annealing data in Figs. 1, 3, and 5. The relative rate of annealing is determined by comparison of the $C_0t_{1/2}$ of DNase-treated samples with the $C_0t_{1/2}$ of total sonicated DNA with globin (△), ovalbumin (○), and rare sequence (●) cDNAs. The percent DNase I digestion reflects the level of acid solubilization of total nuclear DNA.

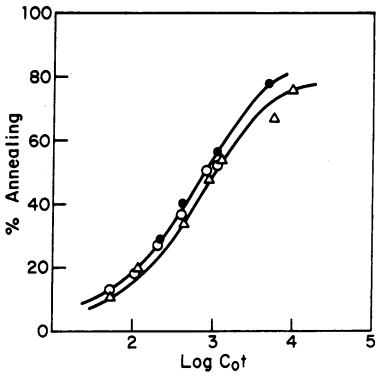

FIG. 3. Kinetics of annealing of globin cDNA with DNase I-treated oviduct nuclear DNA. Globin [^{3}H]cDNA (0.12 ng) was annealed with 2.4 mg of sonicated total DNA (●) and DNA extracted from DNase I-treated oviduct nuclei after 7% (○) and 24% (△) solubilization of nuclear DNA.

ganized by chromatin protein in a conformation that renders them specifically sensitive to nuclease attack.

As the digestion proceeds, there is a striking reduction in the average molecular weight of the DNA products. The weight average molecular weight of our sonicated total DNA is 250 nucleotides. It was therefore important to verify that the reduction in the rate of reassociation observed after DNase I digestion was not merely the result of a decrease in the size of the annealing species. A comparison of the kinetics of digestion of the globin and ovalbumin genes (Fig. 2) renders this possibility highly unlikely since these experiments were performed with identical preparations of DNase I-treated DNA. An additional control was performed in which oviduct DNA was purified free of chromatin proteins and reconstituted with a purified preparation of bovine histones. This reconstitution permits the reassembly of nucleosomes along the DNA, but will not result in the generation of an active conformation about the ovalbumin genes. This reconstituted complex was then subjected to digestion with DNase I, and the resulting DNA fragments were then annealed with ovalbumin cDNA. A 30% DNase digest shows only a 20% decrease in the rate of annealing to ovalbumin cDNA when compared with total unfractionated oviduct DNA (data not shown), further indicating that the observed decrease

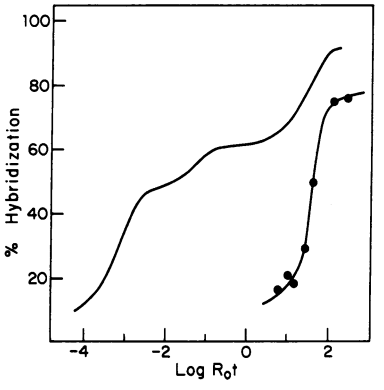

FIG. 4. Hybridization of total and scarce sequence cDNA with oviduct poly(A)-mRNA. The solid curve represents a computer-described best fit to data obtained by annealing cDNA prepared from total oviduct poly(A)-mRNA with the template RNA as published previously (16). The cDNA corresponding to the final transition was purified by several cycles of hybridization. A 0.5-ng sample of this scarce sequence cDNA was annealed with 20 μg of poly(A)-mRNA in a volume of 50 μl (●). Aliquots were removed at various times and assayed with S_1 nuclease.

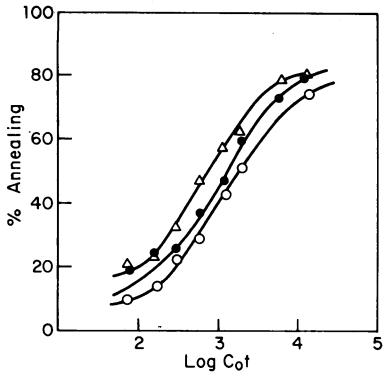

FIG. 5. Kinetics of annealing of oviduct scarce sequence cDNA to DNase I-treated nuclear DNA. cDNA complementary to the complex, rare class of oviduct polysomal RNA was purified from cDNA synthesized from total poly(A)-containing polysomal RNA by several cycles of hybridization. A 0.6-ng sample of rare sequence [^{3}H]cDNA was annealed with DNA extracted from DNase I-treated oviduct nuclei after 3% (△), 11%, (●), and 24% (○) solubilization of nuclear DNA.

in the rates of annealings does not merely result from a nonspecific reduction in the size of the annealing fragments.

Accessibility of scarce mRNA genes in chromatin

About 100,000 copies of ovalbumin mRNA are present in each tubular gland cell of the oviduct. In contrast, the scarce mRNA sequences are represented by only five copies of each sequence per cell (16). Whatever mechanisms are invoked to explain the maintenance of these steady-state mRNA levels, it is likely that the enormous difference in the representation of these two subsets of gene sequences results at least in part from large differences in the rates of initiation of transcription. It is therefore probable that the distribution of polymerases on those genes represented infrequently in the oviduct cell should be significantly lower than the distribution of polymerases required to maintain a steady-state level of ovalbumin RNA synthesis. Examination of the accessibility of these scarce mRNA sequences in chromatin to DNase I attack should therefore permit us to assess the possible role of RNA polymerase in the maintenance of an active conformation about transcriptionally active genes.

These experiments initially required that we isolate a purified cDNA preparation that is a copy of the complex mRNA and that is free of ovalbumin sequences. The annealing of cDNA prepared with total poly(A)-mRNA from the oviduct as template with the template mRNA preparation is shown in Fig. 4. The kinetic profile is clearly complex, and computer analysis reveals that a best fit is obtained for three discrete frequency classes in the oviduct mRNA (16). The final transition anneals with an observed R_0t of 30 and represents the scarce, complex class of 14,000 different sequences. To obtain a purified preparation of cDNA complementary to this class of RNA, we hybridized total cDNA with oviduct poly(A)-mRNA to an R_0t value of 2, which will permit the reassociation only of cDNA complementary to the more abundant species of mRNA. Sixty-five percent duplex formation was obtained at this R_0t value. The reaction was then fractionated by hydroxylapatite chromatography to obtain the cDNA that had not yet reassociated. This single-stranded cDNA was purified free of RNA and allowed to anneal to an excess of oviduct poly(A)-mRNA to an R_0t value of 200. The cDNA reassociated at this R_0t was isolated and again purified free of RNA. In Fig. 4 we observe that this cDNA now anneals as single first-order transition with an $R_0t_{1/2}$ of 35, consistent with the fact that it is a copy of the most complex, least abundant class of oviduct mRNA. Less than 5% of this cDNA anneals to purified preparations of ovalbumin mRNA. These annealing data provide strong support for the existence of a rather discrete frequency class of mRNA of low abundance and indicate that the cDNA that we have obtained through several cycles of hybridization accurately reflects this class of mRNA and is present in relatively pure form.

This scarce sequence cDNA was now annealed with DNase I-treated oviduct DNA preparations used in the previous experiments shown in Figs. 1 and 3. The kinetics of reassociation of this cDNA with total unfractionated oviduct DNA reveals a second-order transition with a $C_0t_{1/2}$ of 520, a value similar to that obtained with ovalbumin cDNA. We again observe with DNase I preparations that, as the digestion proceeds, the relative rate of annealing of this scarce sequence cDNA diminishes with increasing extents of digestion (Fig. 5). A limiting C_0t value of 1900, a value 3.3 times that observed with total DNA, is obtained at 24% solubilization of nuclear DNA. It is apparent from Figs. 2 and 5 that these scarcely represented sequences are maintained in a conformation that is as accessible to nuclease digestion as is the rapidly synthesizing ovalbumin gene. These data would therefore indicate that the mere presence of polymerase about a given sequence is not sufficient to render it selectively sensitive to DNase I attack.

DISCUSSION

Analysis of the products of digestion of chromatin reveals a set of characteristic nucleoprotein complexes that reflect an aspect of structure of the total genome. In our studies we have probed the DNA generated upon nuclease treatment of chromatin with cDNA copies of specific mRNA sequences to study the structure and organization of transcriptionally active genes in the chromosome. In initial studies we demonstrated that the transcriptionally active ovalbumin gene in the oviduct is associated with chromatin proteins to generate a structure with nuclease-sensitive sites at 200-base-pair intervals along the DNA (3, 6). These results strongly suggest that histone molecules are present about the ovalbumin gene during transcription. Our results, however, do not permit us to comment on the degree of compaction of the ovalbumin genes. Recent electron microscopic studies have revealed that while nucleosomes are present along transcriptionally active genes, the packing ratio about these segments of the genome is significantly lower than that observed about the bulk of transcriptionally inert chromosomes (17, 18). Other studies have failed to detect nucleosomes about transcription units, but observe extended chromatin fibers between nascent ribonucleoprotein fibrils (23). It is therefore likely that active genes exist in an extended or unfolded conformation that still maintain the periodic nuclease-sensitive sites characteristic of the bulk of the genome.

In accord with this prediction, we find that the ovalbumin genes in the oviduct appear to be organized by chromatin proteins in such a way that they are rendered exceedingly sensitive to digestion by DNase I. This sensitivity is not observed in the liver, a tissue in which these genes are transcriptionally inert. Furthermore, the transcriptionally inactive globin genes in the oviduct are not selectively sensitive to nuclease attack and are digested 5 times more slowly than the ovalbumin genes in this tissue. The converse is true for embryonic erythroblasts, where the globin genes in chromatin, but not the ovalbumin gene, are preferentially digested by DNase I (2). These studies indicate that some component of the chromatin complex is responsible for maintaining transcriptionally active segments of the chromosome in a conformation that renders them ac-

cessible to attack by DNase I only in those tissues in which these genes are transcribed.

These experiments provide little information on the nature of those components of the chromatin complex responsible for the maintenance of this active conformation about transcriptionally active segments of the chromosome. It is possible that the sensitivity of these genes to DNase I attack reflects the distribution of polymerase about these regions of the chromosome. To this end, the sensitivity of active gene sets represented at widely differing frequencies within the mRNA population were compared. About half of the mRNA present in the fully induced oviduct consists of ovalbumin mRNA sequences. Measurement of the initiation frequency required to maintain a steady-state level of ovalbumin mRNA observed in the induced oviduct require 10–20 initiations per min along this gene (19). This initiation rate results in a high density of polymerase molecules about the ovalbumin gene corresponding to one polymerase molecule per 200 base pairs of DNA. It is difficult at present to determine accurately the rate of transcription of those genes coding for the more complex, scarce mRNA. Each of these sequences is present 20,000 times less frequently than ovalbumin mRNA in the oviduct cytoplasm. Our assumption that the distribution of polymerases about these genes is significantly lower than that present about the ovalbumin gene is based on the following observations: (*i*) If mRNA sequences are conserved, then maintenance of the steady-state level of this complex class of mRNAs in the oviduct would only require initiation frequencies of 5–10 initiations per day, a value several thousandfold lower than that observed for the ovalbumin gene. (*ii*) If 10% of the genome is transcribed in the oviduct and the number of available polymerase B molecules per oviduct nucleus is 1 to 3×10^4 (20, 21), the maximum distribution of polymerase averages to be one enzyme per 4000 base pairs. This value is 20 times lower than that required to maintain the steady level of ovalbumin RNA in the oviduct. (*iii*) Where measured, as in the sea urchin embryo, the transcription rate for this scarce subset of mRNA sequences is estimated to be two per day (22). We conclude, therefore, that the widely differing frequencies of these two subsets of genes in the oviduct result at least in part from the strikingly different rates of transcription.

Analysis of the conformation of these two gene sets in chromatin with DNase I reveals that they are recognized and cleaved by this enzyme at similar rates. The mere presence of polymerase along a given gene therefore does not seem to be responsible for the maintenance of an active conformation. This conclusion must be tempered by the possibility that the ovalbumin gene may be far more accessible to nucleolytic attack than the rare sequence genes but the dense concentration of polymerase along this gene inhibits DNase I action, thus equalizing the observed digestion rates.

Finally, it is unlikely that a significant proportion of these rare sequence genes would be actively transcribed at the moment of sampling. The DNase-sensitive conformation therefore appears to be maintained between transcriptional events. These data are in accord with observations in mature erythrocytes which demonstrate specific sensitivity of the globin genes to DNase I attack long after transcription of these gene has ceased (2). These results suggest that the maintenance of an active conformation about specific genes does not reflect the polymerase distribution about these genes. This conformation is therefore not confined to sequences actively engaged in the transcription process and may reflect the structure about a subpopulation of the genome which represents the transcriptional potential of a given cell type.

We thank Dr. Sol Spiegelman for helpful discussion and critical reading of this manuscript. In addition, we thank Drs. Pat Williamson and Gary Felsenfeld for kindly providing the chicken globin cDNA used in these experiments. This investigation was supported by Grant CA-16346, awarded by the National Cancer Institute, Department of Health, Education, and Welfare.

1. Lacy, E. & Axel, R. (1975) *Proc. Natl. Acad. Sci. USA* 72, 3978–3982.
2. Weintraub, H. & Groudine, M. (1976) *Science* 193, 848–856.
3. Garel, A. & Axel, R. (1976) *Proc. Natl. Acad. Sci. USA* 73, 3966–3970.
4. Gottesfeld, J., Murphy, R. F. & Bonner, J. (1975) *Proc. Natl. Acad. Sci. USA* 72, 4404–4408.
5. Tien Kuo, M., Sahasrabuddhe, C. G. & Saunders, G. F. (1976) *Proc. Natl. Acad. Sci. USA* 73, 1572–1575.
6. Axel, R. & Garel, A. (1976) *Biochemical Actions of Progesterone* (New York Academy of Science, New York), Vol. 286, 135–146.
7. Daneholt, B. (1975) *Cell* 4, 1–10.
8. Axel, R., Cedar, H. & Felsenfeld, G. (1973) *Proc. Natl. Acad. Sci. USA* 70, 2029–2032.
9. Gilmour, R. S. & Paul, J. (1973) *Proc. Natl. Acad. Sci. USA* 70, 3440–3442.
10. Jacquet, M., Groner, Y., Monroy, G. & Hurwitz, J. (1974) *Proc. Natl. Acad. Sci. USA* 71, 3045–3049.
11. Harris, S. E., Schwartz, R. J., Ming-Jer, T. & O'Malley, B. W. (1976) *J. Biol. Chem.* 251, 524–529.
12. Gjerset, R. A., Bressmann, H., Levy, B. & McCarthy, B. J. (1976) *ICN-UCLA Symp.* V, 279–307.
13. Levy, B. & Dixon, G. H. (1977) *Nucleic Acids Res.* 4, 883–898.
14. Weintraub, H., Worcel, A. & Alberts, B. (1976) *Cell* 9, 409–417.
15. Schutz, G., Kieval, S., Groner, B., Sippel, A., Kurtz, D. T. & Feigelson, P. (1977) *Nucleic Acids Res.* 4, 71–84.
16. Axel, R., Feigelson, P. & Schutz, G. (1976) *Cell* 7, 247–254.
17. McKnight, S. & Miller, D. L. (1976) *Cell* 8, 305–319.
18. Foe, V., Wilkinson, L. & Laird, C. (1976) *Cell* 9, 131–146.
19. Palmiter, R. D. (1974) *Cell* 4, 189–197.
20. Cochet-Meilhac, M., Nuret, P., Courvalin, J. C. & Chambon, P. (1974) *Biochim. Biophys. Acta* 353, 185–192.
21. Cox, R. F. (1976) *Cell* 7, 455–465.
22. Galau, G. A., Lipson, E. D., Britten, R. J. & Davidson, E. H. (1977) *Cell* 10, 415–432.
23. Franke, W. W., Scheer, U., Trendelenburg, M. F., Spring, H. & Zentgraf, H. (1976) *Cytobiologie* 13, 401–434.