Proc. Nat. Acad. Sci. USA
Vol. 72, No. 10, pp. 3978–3982, October 1975
Biochemistry

Analysis of DNA of isolated chromatin subunits

(nuclease/DNA reassociation/DNA electrophoresis)

ELIZABETH LACY AND RICHARD AXEL

Institute of Cancer Research and Department of Pathology, Columbia University, College of Physicians & Surgeons, New York, N.Y. 10032

Communicated by Jacob Furth, August 13, 1975

ABSTRACT **Partial digestion of rat liver nuclei with staphylococcal nuclease results in the liberation of nucleoprotein complexes consisting of one or more ν bodies. By velocity centrifugation we have isolated the monomeric subunit in relatively pure form. We find that this subunit contains 185 base pairs of DNA and 240,000 daltons of protein, resulting in a protein to DNA ratio identical to that of unperturbed chromatin. The isolated monomeric particle is further susceptible to internal nuclease attack resulting in the solubilization of 46% of the monomeric DNA. Analysis of the resistant DNA reveals a complex but highly reproducible pattern of DNA fragments ranging from 160 to 60 base pairs in length. Analysis of the reassociation kinetics of the isolated subunit DNA reveals that most, if not all genomic sequences, are involved in this basic subunit structure. No special frequency class of DNA is absent from ν bodies. Furthermore, virtually all liver sequences transcribed into mRNA are present in ν body DNA. These results indicate that ν body formation may be random with respect to DNA sequence and suggest that the mere presence of ν bodies over a specific region of DNA is not sufficient to restrict its transcription.**

Over the past year an experimentally consistent model of chromatin structure has emerged which postulates the presence of regularly repeating nucleoprotein subunits joined by short segments of DNA. Biochemical evidence for a periodic protein–DNA complex was initially introduced by Hewish and Burgoyne (1) and subsequently confirmed in several laboratories (2–6). These data demonstrate that mild nuclease digestion of nuclei results in the liberation of a series of nucleoprotein particles, ν bodies, containing DNA fragments whose molecular weights are all multiples of a unit fragment 150–200 base pairs in length. Subsequent studies (4, 5, 7, 8) have revealed that the ν body represents a transient intermediate in the digestion process and that cleavage of DNA within the ν body results in the generation of a true limit digest reflecting the internal structure of the monomeric subunit.

Electron microscopic observations (9, 10) are in accord with the biochemical data and reveal the existence of spherical nucleoprotein particles which are joined by a short filament of DNA. These particles can be observed closely apposed to one another in gently disrupted nuclei, providing convincing evidence that these nucleoprotein particles reflect a level of structure characteristic of the chromatin fiber *in vivo*.

The elucidation of a subunit structure in chromatin immediately poses the question as to the possible role of this structure in those biological processes in which chromatin participates. In this report we describe procedures for the isolation and characterization of highly purified ν bodies. We find that most if not all of the DNA sequences in the genome are involved in the formation of these particles. No specific frequency class of DNA is restricted from particle formation. Further, virtually all sequences expressed as mRNA in liver appear to be present within the DNA of isolated ν bodies. The template active portion of the genome is therefore not exempt from involvement in the basic repeating structure.

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

MATERIALS AND METHODS

Nuclease Digestion of Chromatin, Nuclei, and Monomer. Nuclei were obtained from rat livers by homogenization and washing with sucrose–triton buffers (11). Chromatin was obtained from intact nuclei by a stepwise reduction in ionic strength (11). Chromatin or monomeric DNA was digested with staphylococcal nuclease (Worthington Biochemical Corp.) in 1 mM Tris·HCl at pH 7.9, 0.1 mM $CaCl_2$ as described (7). Digestion of nuclei was carried out on suspensions of nuclei washed twice in 1 mM Tris·HCl at pH 7.9, 0.1 mM $CaCl_2$, 0.25 M sucrose and resuspended in the same buffer at a DNA concentration of 150 μg/ml. After the addition of nuclease, the kinetics of DNA digestion was assayed by measuring the amount of A_{260} absorbing material soluble in 1 M NaCl, 1 M $HClO_4$. Nuclease reactions were terminated by the addition of NaEDTA to 5 mM. Resistant DNA was purified by treatment with proteinase K (E. Merck) and phenol extraction as described (7).

Preparation of Monomer. Purified nuclei were suspended and washed in 0.01 M Tris·HCl at pH 7.9, 0.1 mM $CaCl_2$, 0.15 M sucrose. Nuclei were resuspended at a concentration of 1 mg/ml and digested with staphylococcal nuclease (1.5 μg/ml) at 37° to yield about 10% digestion of DNA. The reaction was terminated by the addition of NaEDTA to 5 mM. The suspension was then layered on a 5–20% linear sucrose gradient in 5 mM NaEDTA at pH 7 and centrifuged 16 hr at 25,000 rpm in an SW27 rotor at 4°. Fractions corresponding to monomer were pooled and dialyzed and concentrated by Amicon ultrafiltration. This crude monomeric fraction was further purified by a second sucrose velocity centrifugation.

Gel Electrophoresis. Electrophoresis of DNA was performed in either 3 or 6% polyacrylamide slab gels. Three percent gels were supplemented with 0.5% agarose to provide greater support. The buffer system employed was that described by Peacock and Dingman (12), and the electrophoresis was carried out at 200 V on a 17 cm cooled slab gel. Gels were stained overnight in "Stains-all" (Eastman), 0.005% in 50% formamide, destained in water, and photographed.

Preparation of DNA. Total rat liver DNA was prepared from isolated nuclei as described (11). This DNA was sonicated (Heat Systems—Ultrasonics Inc. model W185) in 0.1 M NaOH to a weight average degree of polymerization of 300 base pairs. Total DNA labeled with ^3H was prepared in

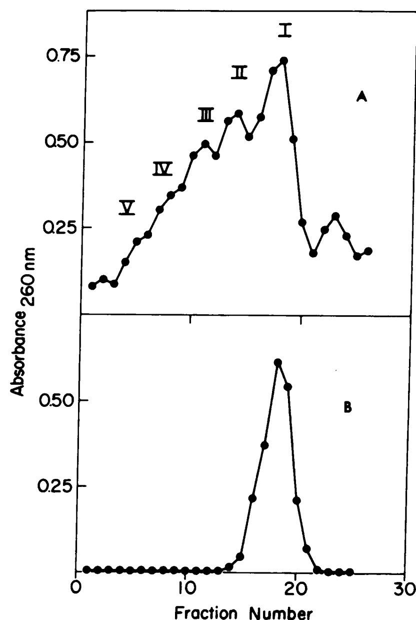

FIG. 1. Sucrose gradient centrifugation of chromatin subunits: the nucleoprotein particles generated by a 4% digestion of rat liver nuclei were analyzed by sucrose gradient centrifugation as described in *Materials and Methods*. The direction of sedimentation proceeds from right to left (A). Fractions corresponding to peak I were pooled, and dialyzed and concentrated by Amicon ultrafiltration. This fraction was resedimented in a second sucrose velocity gradient (B).

an identical fashion from cultures of rat liver cells labeled for 36 hr with [^{3}H]thymidine (20 μCi/ml). DNA prepared in this way had a specific activity of 40,000 cpm/μg.

Synthesis of cDNA. mRNA that contained poly(A) was prepared from purified rat liver polysomes as previously described (13). Globin 9S mRNA was purified from duck reticulocyte polyribosomes by oligo(dT) cellulose chromatography and sucrose gradient centrifugation. [^{3}H]DNA copies complementary to rat liver mRNA and duck globin mRNA were synthesized with RNA-dependent DNA-polymerase purified from avian myeloblastosis virus (14–16).

Annealings. Annealings were performed in 1 mM Tris·HCl at pH 7.9, 0.1 mM EDTA, 0.4 M NaCl at 69° under paraffin oil. Aliquots were removed and diluted in 0.5 ml of 0.03 M NaAc at pH 4.5, 0.12 M NaCl, 1.2 × 10^{-4} M $ZnSO_4$; 0.25 ml was immediately precipitated with trichloroacetic acid and the other half was incubated at 37° for 1 hr in the presence of S_1 nuclease. The amount of radioactive DNA resistant to digestion was determined by trichloroacetic acid precipitation.

RESULTS

Isolation and characterization of purified ν bodies

Characterization of the basic repeat unit of chromatin requires that we isolate the monomeric particle in relatively pure form. Early in the digestion of intact nuclei with the enzyme staphylococcal nuclease, a series of nucleoprotein fragments is liberated. The fragments are multimeric complexes consisting of one or more ν bodies. These structures are readily separable from one another by sucrose gradient velocity centrifugation (2, 5). When rat liver nuclei are partially digested with nuclease and the resulting fragments sedimented in sucrose gradients, the profile shown in Fig. 1A is obtained. This pattern results from only 4% digestion of nuclear DNA and reveals at least five absorbance peaks.

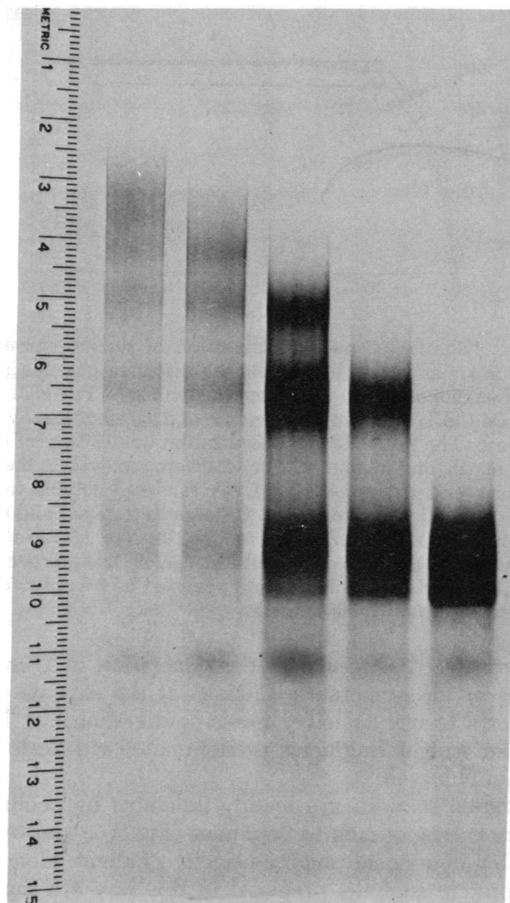

FIG. 2. 3% polyacrylamide slab gel electrophoresis of nuclear digestion products: Fractions corresponding to peaks I–V (Fig. 1A) were pooled and the associated DNA purified by treatment with proteinase K and phenol extraction; 40 μg of DNA were applied to each slot of a 3% polyacrylamide–0.5% agarose slab, electrophoresed at 200 V for 2.5 hr, stained, and photographed. The samples from right to left represent the DNA fragments contained in peaks I–V in Fig. 1A.

Identification of these peaks as multimeric forms of a basic monomeric nucleoprotein fragment requires that we examine the DNA within each complex. To this end, fractions were pooled from the regions of the gradient labeled I–V and the DNA was freed of its associated protein. This DNA was then analyzed by electrophoresis on 3% composite agarose-acrylamide gels (Fig. 2). A broad band is observed in peak I (185 base pairs in length); thus this peak is defined as the monomeric subunit. In addition, this peak reveals a minor component that is 160 base pairs long, which represents the initial degradation product of the ν body. Peak II contains dimeric DNA that is 360 base pairs in length but is still grossly contaminated by the monomeric particle. Fractions III, IV, and V reveal the appearance of additional DNA bands of 550, 730, and 920 base pairs in length. Gels were calibrated by electrophoresis and autoradiography of [^{32}P]DNA fragments obtained by *Hin* restriction endonuclease cleavage of λ DNA (kindly provided by Dr. Tom Maniatis). Although these fractions are poorly resolved from one another, these data still allow us to identify tentatively each of the nucleoprotein peaks as multimeric forms of the basic subunit reflected in peak I.

It should be noted that the profile shown in Fig. 1 results from digestion of only 4% of the DNA in intact nuclei. More

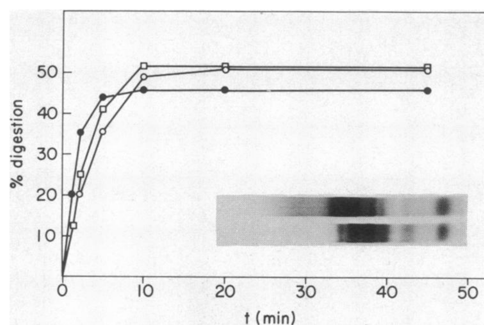

FIG. 3. Kinetics of nuclease digestion of nuclei, chromatin, and monomer: Intact rat liver nuclei (O), isolated chromatin (□), and purified monomer (●) were incubated in 1 mM Tris·HCl at pH 7.9, 0.1 mM $CaCl_2$ at 37° in the presence of 5 μg/ml of staphylococcal nuclease. The initial DNA concentration was 150 μg/ml in nuclei, chromatin, and monomer. Aliquots were removed at the indicated times and the amount of DNA rendered soluble in 1 M $HClO_4$–1 M NaCl was determined. Chromatin (top gel) and monomer (bottom gel) were digested, as described in *Materials and Methods*, to 53% and 46% acid solubilization of DNA, respectively. The resulting DNA was purified and analyzed by 6% polyacrylamide slab gel electrophoresis (insert).

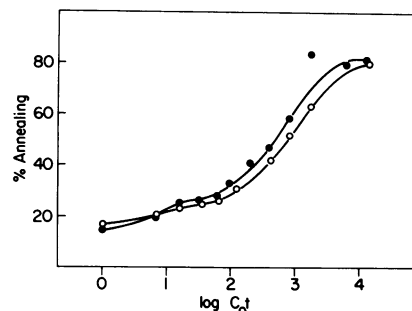

FIG. 4. Kinetics of annealing of rat liver DNA labeled with ^3H to monomeric and total nuclear DNA: 10,000 cpm (0.25 μg) of total rat liver DNA was annealed with 25 or 750 μg of unlabeled total DNA (●) or monomeric DNA (O) in a reaction volume of 30 μl. Aliquots were removed at various times and duplex formation was monitored by S_1 nuclease digestion as described in *Materials and Methods*.

extensive digestion results in the generation of increasing amounts of the monomeric subunits at the expense of the multimeric forms. At lower levels of digestion, the higher molecular weight fragments predominate, with little or no monomer observed.

Individual subunits are readily isolatable by pooling the fractions corresponding to monomer (Fig. 1A) and by resedimenting in a second sucrose velocity gradient. Monomeric nucleoprotein subunits prepared in this way are shown in Fig. 1B. Analysis of the DNA within this single peak by electrophoresis reveals a predominant band of 185 base pairs and a small amount of the 160 base pair component. No other size classes of DNA are represented in this fraction.

These isolated subunits have a protein to DNA ratio of 2:1. By acid extraction we find that 60% of the total protein is histone, resulting in a histone:DNA ratio of 1.2:1. Analysis of the histones on acid-urea gels (17) reveals all five major classes in proportions similar to those observed in undigested chromatin with only a slight reduction in histone F1. A similar pattern is observed with ν bodies isolated from duck reticulocyte nuclei. In this case the protein:DNA ratio of ν bodies is 1.4 with a histone to DNA ratio of 1.1:1. It is therefore probable that the subunits contain acidic protein in amounts analogous to that of intact chromatin. The protein complement of the isolated subunits therefore mirrors the pattern observed in the native chromatin from which the subunits were originally isolated.

A population of fragments consisting almost entirely of monomeric subunits can be generated after only 14% digestion of intact nuclei. The observation that the DNA of either nuclei or purified chromatin is 50% digestible by nuclease (4, 5, 18), immediately suggests that the individual monomeric subunit must contain internal cleavage sites accessible to nuclease attack. The kinetics of digestion of the DNA of the subunits, as well as of chromatin and nuclei, are seen in Fig. 3. Both chromatin and nuclear DNA reach a well defined limit to the digestion process at 53% solubilization of DNA. Digestion of isolated ν bodies is more rapid and results in 46% solubilization at the limit. At the end of the digestion process, the resulting nucleoprotein fragments precipitate. These fragments contain half of the original complement of DNA but all of the initial monomeric proteins, resulting in a protein to DNA ratio of about 4. No proteins appear to be released free into solution during the digestion process. Furthermore, digestion of monomeric particles, which have been crosslinked with formaldehyde (19) to prevent protein exchange, reveals an identical limit at 46% digestion of DNA.

The DNA resulting from limit digestion of either rat liver chromatin or isolated ν bodies was analyzed by 6% polyacrylamide gel electrophoresis (Fig. 3, insert). The gel pattern reveals a set of eight reproducible DNA fragments ranging in size from 160 to 60 base pairs. Whereas, differences in intensity exist for the fragments, the molecular weights of the resistant DNA from the two sources are identical. The DNA fragments obtained upon limit digestion of either intact nuclei or chromatin therefore result from cleavage at specific sites within the ν body.

Kinetic complexity of ν body DNA

The availability of purified ν bodies now permits us to characterize the DNA sequences present within the monomeric subunit. Perhaps the most direct experimental approach to this problem involves an analysis of the reassociation kinetics of isolated monomeric and total nuclear DNA. To this end, tritium labeled total nuclear DNA was prepared from cultures of normal rat liver cells. This DNA was then annealed in the presence of a vast excess of either total or isolated ν body DNA. The reassociation of total DNA labeled with ^3H to a vast excess of cold rat liver DNA is seen in Fig. 4. As expected, two transitions are observed over the range of C_0t values studied. Thirty percent of the DNA consists of repeated sequences. The final transition reflects the reassociation of the unique sequences of the rat genome which have a $C_0t_{1/2}$ of 820.

A similar pattern is obtained when total DNA labeled with ^3H is annealed with DNA isolated from purified ν bodies; 28% of the ν body DNA consists of repeated sequences. The unique sequences reassociate with total DNA labeled with ^3H with a $C_0t_{1/2}$ of 1000. Both reactions saturate at 83% duplex formation. This concordance indicates that virtually all sequences present in the rat genome are represented in the ν body DNA.

The difference in the rate of reassociation of the two DNAs is probably due to the different average molecular weights of the DNA fractions. Total DNA is sonicated to a weight average degree of polymerization of 300 base pairs, whereas monomer is 185 base pairs in length. Theoretically, the larger total DNA should reassociate 1.3 times faster than

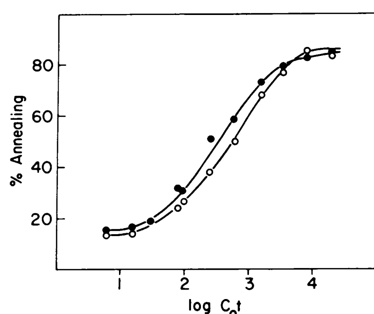

FIG. 5. Kinetics of annealing of rat liver cDNA labeled with ^{3}H to monomer and total rat liver DNA: cDNA copies of rat liver mRNA were prepared as described in *Materials and Methods.* 10,000 cpm (0.5 ng) of cDNA was annealed with 750 μg of either total DNA (●) or monomeric DNA (○). Annealing reactions were performed in a volume of 30 μl and duplex formation was monitored with S_1 nuclease.

monomeric DNA (20). We find that the unique sequences of total DNA reassociate 1.25 times faster than monomeric DNA. It would appear then that all genomic sequences are represented in the ν body and that the reiteration frequency of these sequences is identical in the two DNA populations.

Transcribed sequences in ν body DNA

The previous annealings do not exclude the possibility that a small but relevant subclass of sequences are excluded from monomeric DNA. Candidates for such a population of DNA are those sequences actively transcribed within the genome. An obvious approach to this problem simply involves probing ν body DNA by molecular hybridization for those sequences present as mRNA in the liver cell. mRNA that contains poly(A) was therefore purified from liver polyribosomes and cDNA copies of this RNA were synthesized with reverse transcriptase isolated from avian myeloblastosis virus (14–16). This cDNA was then used as a probe in annealing reactions to detect the presence of expressed sequences in total and monomeric DNA. The kinetics of annealing of highly radioactive cDNA to these two populations of DNA is shown in Fig. 5. The annealing curves for both DNAs are quite similar. The reaction reaches saturation with 85% of the cDNA involved in duplex formation. This indicates that virtually all of the transcribed sequences reflected in cDNA are present within the basic chromatin subunit. From the kinetics of annealing we observe that most of the sequences in cDNA are transcribed by the unique sequences of the genome. About 10% of the cDNA has annealed to more highly reiterated DNA, a finding consistent with recent work from other laboratories (21). The small difference in the rate of annealing of cDNA to total nuclear and monomeric DNA can again be attributed to the sizes of the two DNAs. These data indicate that the relative frequency of transcribed sequences is identical in both total and ν body DNA. This suggests that formation of a monomeric subunit over a particular segment of DNA is not sufficient to inhibit transcription of this sequence.

An alternate approach to the question of whether transcribed sequences participate in ν body structure involves the quantitation of specific genes in ν body DNA. Perhaps the system most amenable to this sort of analysis is that of globin RNA synthesis by immature avian reticulocytes. In these experiments, a fixed amount of globin cDNA is titrated with increasing quantities of either total nuclear or monomeric DNA under conditions that allow completion of the

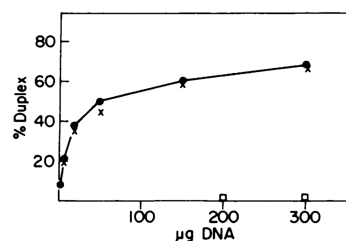

FIG. 6. Titration of globin genes in monomeric and total DNA of duck reticulocytes: 800 cpm (0.03 ng) of globin cDNA was anwas annealed with increasing quantities (5–300 μg) of either monomeric (×) or total (●) reticulocyte DNA in a volume of 25 μl. Reactions were incubated at 69° for 96 hr and assayed by treatment with S_1 nuclease. □ = *Escherichia coli* DNA.

annealing reactions (22). From the initial slope of the titration curve, we determine what proportion of the cDNA is annealed to a known quantity of cold genomic DNA. The concentration of the globin genes in a population of DNA can therefore be determined directly from these measurements. This approach only assumes that the cDNA and the corresponding genomic minus strand share the same rate constant of reaction to the natural plus strand.

The titration of 0.2 ng of duck globin cDNA with total or ν body DNA of reticulocytes is seen in Fig. 6. The curves for the two DNA fractions are identical and demonstrate that the reiteration frequency of the globin genes is the same in monomer as it is in total DNA, even in a tissue actively expressing these sequences. From the titrations we calculate the presence of two copies of each of the globin genes per haploid genome, a finding consistent with previous work from other laboratories (23, 24).

From these data it appears that ν bodies are formed over sequences actively transcribed in chromatin. These studies do not rule out the possibility that a class of repressed sequences are absent from ν body DNA. Preliminary experiments, however, reveal that the concentration of globin genes in monomeric DNA derived from avian erythrocytes is again identical to that observed in total DNA. This would suggest that repression of a given sequence cannot be equated with restriction of that sequence from ν body formation.

DISCUSSION

These studies, as well as those in other laboratories, reveal that the interphase eukaryotic chromosome consists of a series of regularly repeating nucleoprotein subunits. It seems reasonable to assume that the particles obtained by mild nuclease digestion of nuclei are identical to the spherical bodies observed in electron micrographs. The monomeric particle, however, represents a transient intermediate in the digestion process. Further digestion of isolated subunits releases 46% of the DNA as acid soluble oligomers. The remaining DNA reveals a series of discrete fragments that range in size from 160 to 60 base pairs and represent specific cleavage sites for nuclease within the monomeric particle.

Careful analysis of the kinetics of digestion, therefore, allows us to discern two levels of structure within the chromatin complex. The initial site of nuclease attack in chromatin appears to reside within the filaments of DNA bridging adjacent ν bodies. Less accessible sites exist within the monomeric subunit and release DNA fragments which probably represent the points of intimate association of histones with DNA.

From the number and sizes of the DNA fragments obtained upon limit digestion of monomeric subunits, it is

probable that more than one repeating subunit exists (7, 8). This heterogeneity may be generated by different arrangements of histones or groups of histones along the DNA or by various conformations of a fixed complement of histones. Alternatively, the heterogeneity may result from the association of acidic proteins with individual ν bodies.

The reassociation of radioactive total nuclear DNA to DNA from isolated ν bodies demonstrates that virtually all genomic sequences are involved in the repeat structure. No specific frequency class of DNA seems to be deleted from the ν body. Furthermore, preliminary studies in our laboratory indicate that satellite sequences are present in mouse liver ν bodies in relative concentrations identical to that of total DNA. These results are consistent with earlier observations that at least 80% of the nuclear DNA can be isolated as monomer after short periods of digestion (2, 4, 5). These data suggest that no long special sequence of nucleotides is required for ν body formation and that the distribution of ν bodies may be random with respect to DNA sequence. This is in accord with our previous experiments demonstrating that equivalent limit digest patterns can be generated from reconstituted nucleoprotein by either eukaryotic, bacterial, or phage DNA (7). In addition, studies on the complexity of this resistant DNA reveal that the entire genome is represented in this "covered" DNA fraction, further suggesting the random arrangement of histone with the vast majority of genomic DNA (25).

Perhaps more revealing are the annealing studies with cDNA which demonstrate that the frequency of sequences transcribed as mRNA are the same in ν body and total nuclear DNA. This is true as well for the globin genes in monomeric subunits from avian reticulocytes. The template active regions of the genome therefore participate in the basic repeat structure characteristic of chromatin.

If some of the proteins of chromatin do act as transcriptional control factors, then we would expect that proteins must exist which are capable of recognizing specific regulatory sites within the chromosome. Our results suggest that 40% of the monomeric protein is non-histone. Since the repeating subunit structure can be reconstituted with DNA and histones alone (7, 10), we can assume that the acidic proteins are probably not essential for the assembly and maintenance of ν body structure. If the acidic proteins serve as regulatory factors in gene expression, it is possible that they exert their effect by recognizing specific sites within a preformed nucleoprotein subunit.

We wish to thank Dr. Tom Maniatis for providing restriction fragments of λ DNA and Dr. I. B. Weinstein for providing us with cultures of rat liver cells. The generous support and helpful criticism of Dr. Sol Spiegelman are gratefully acknowledged. This work was supported by the National Institutes of Health, National Cancer Institute, Research Grant CA-16346.

1. Hewish, D. R. & Burgoyne, L. A. (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**, 2915–2925.
5. Sollner-Webb, B. & Felsenfeld, G. (1975) *Biochemistry* **14**, 2916–2921.
6. Kornberg, R. & Thomas, J. O. (1974) *Science* **184**, 865–868.
7. Axel, R., Melchior, W., Sollner-Webb, B. & Felsenfeld, G. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 4101–4105.
8. Weintraub, H. & Van Lente, F. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 4249–4253.
9. Olins, A. L., Carlson, R. D. & Olins, D. E. (1975) *J. Cell Biol.* **64**, 528–537.
10. Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Cell* **4**, 281–300.
11. Axel, R., Cedar, H. & Felsenfeld, G. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2029–2032.
12. Peacock, A. & Dingman, C. (1967) *Biochemistry* **6**, 1818–1827.
13. Schutz, G., Killewich, L. & Feigelson, P. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 1017–1020.
14. Kacian, D. L., Speigelman, S., Bank, A., Terada, M., Metafora, S., Dow, L. & Marks, P. A. (1972) *Nature New Biol.* **235**, 167–169.
15. Verma, J. M., Temple, G. F., Fan, H. & Baltimore, D. (1972) *Nature New Biol.* **235**, 163–167.
16. Ross, J., Aviv, H., Scolnick, E. & Leder, P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 264–268.
17. Panyim, S. & Chalkley, R. (1969) *Biochemistry* **8**, 3972–3979.
18. Clark, R. J. & Felsenfeld, G. (1971) *Nature New Biol.* **229**, 101–106.
19. Brutlag, D., Cameron, S. & Bonner, J. (1969) *Biochemistry* **8**, 3214–3218.
20. Wetmur, J. & Davidson, N. (1968) *J. Mol. Biol.* **31**, 349–370.
21. Bishop, J. O., Morton, J. G., Rosbash, M. & Richardson, M. (1974) *Nature* **250**, 199–204.
22. Young, B. D., Harrison, P. R., Gilmour, R. S., Birnie, G. D., Hell, A., Humphries, S. & Paul, J. (1974) *J. Mol. Biol.* **84**, 555–568.
23. Bishop, J. O. & Freeman, K. B. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **38**, 707–716.
24. Packman, S., Aviv, H., Ross, J. & Leder, P. (1972) *Biochem. Biophys. Res. Commun.* **49**, 813–819.
25. Axel, R., Cedar, H. & Felsenfeld, G. (1975) *Biochemistry* **14**, 2489–2495.