Transcription of the nonrepeated fraction of "accessible" DNA in rat liver chromatin.

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ABSTRACT

The relationship between structure and function in eukaryotic chromatin has been studied in rat liver cells. To elucidate the functional significance of "accessible" DNA, the transcription of this DNA (prepared by titration of liver chromatin with poly-D-lysine) has been examined by RNA-DNA hybridization. The maximum extent to which nuclear RNA will hybridize to the nonrepeated fraction of "accessible" DNA has been measured and compared to the extent that whole chromatin DNA will hybridize. The results show that "accessible" DNA has the same number of sequences complementary to nuclear RNA as does total DNA. In addition DNA-DNA reassociation experiments indicate that there is only a small difference between the total unique sequence populations of "accessible" and total DNA. These results indicate that nonrepeated "accessible" DNA is not preferentially transcribed in the cell as is predicted by some models of chromatin structure.

INTRODUCTION

Although the DNA in mammalian chromatin is found complexed with large amounts of protein, as much as 50% of the DNA phosphate will bind added polylysine as well as other cationic molecules (1,2). The nature of the binding sites themselves is a matter of controversy. It has been proposed by some (1-6) that polylysine binds to specific regions of "open" or "accessible" DNA present in chromatin. These open regions may consist of long stretches of protein free DNA (1,6) or stretches of DNA that are only weakly bound to protein (2,4,5). Others (7,8) have suggested that polylysine may bind in the minor groove of the DNA helix because the majority of the chromosomal proteins may occupy only the major groove.

The functional significance of accessible DNA is also not known but it was originally proposed that genes which are actively transcribed in the cell are located in accessible DNA. This proposal that accessible DNA will be transcribed because the DNA is free to interact with RNA polymerase and other molecules of transcription while inaccessible DNA will be repressed because of the protective cover of tightly bound chromosomal

protein is attractive because of its simplicity. It also suggests experimental designs to test its validity. The experimental evidence up to this point, however, has been inconclusive with some findings lending support to the theory and others arguing against it.

Evidence in support of this model has come from recent studies on the fractionation of chromatin. Results from our own laboratory (9) as well as others (10) have shown that "template-active" fractions of isolated chromatin bind more polylysine than "template-inactive" fractions. Preferential binding of polylysine to template-active chromatin has also been recently demonstrated in cytological preparations (11).

Evidence against this model has come from two observations. In the first place the amount of accessible DNA in chromatin far exceeds the amount of DNA which is actually transcribed in the cell. In most mammalian chromatins 50% of the total DNA is "accessible" but it has been estimated that no more than 5-10% of the total DNA in a specific tissue is actually transcribed into RNA (12). In the second place, there seems to be no correlation between the amount of accessible DNA in the chromatin of a specific tissue and the RNA transcriptional activity of that tissue. For example, both liver and erythrocyte chromatin have equal amounts of accessible DNA (13).

In an earlier paper (14) we described the isolation of accessible DNA (formerly called "polylysine-binding DNA") from rat liver chromatin utilizing poly-D-lysine. In this paper we report the results of experiments designed to test whether or not this accessible DNA fraction is preferentially transcribed in the cell. We have looked only at the non-repeated fraction of the DNA because: 1) this fraction is thought to be made up of the sequences which actually code for the amino acid sequences of protein, and 2) we avoid the interpretational difficulties which arise in hybridization experiments involving the transcripts of repeated DNA sequences or closely-related families of sequences which may be interspersed throughout the genome. In this study we have measured the percentage of the nonrepeated fraction of accessible DNA which will hybridize with liver nuclear RNA and compared the results with the percentage of the non-repeated fraction of whole chromatin DNA that will hybridize. The results of this determination should indicate whether or not accessible DNA is enriched in DNA transcribed in vivo.

MATERIALS AND METHODS

The following materials were purchased commercially: adult male

Sprague-Dawley rats (250-300g) from Holtzman or Charles River; Poly-D-lysine hydrobromide (avg. MW = 70,000) from Sigma Chemical Co.; Hydroxyapatite ("regular grade Bio-Gel HTP") from Bio-Rad Laboratories; S₁ nuclease from Miles Laboratories; wheat germ ribosomal RNA from Cal Biochem; and Na¹²⁵I (carrier free) from New England Nuclear.

Preparation of Chromatin: Rat liver chromatin was prepared from adult Sprague-Dawley rats by extracting purified liver nuclei with saline-EDTA as described previously (9). The chromatin was purified by washing in buffers of low ionic strength and sonicated briefly as described (9).

Isolation of Accessible DNA: Accessible DNA was isolated from chromatin by utilizing poly-D-lysine binding as described previously (14). In short poly-D-lysine-HBr (avg. MW = 70,000) was added to a dilute solution of chromatin until the accessible DNA was completely saturated with the polycation. Chromosomal proteins were then removed by digestion with pronase, and all DNA not bound to polylysine was removed by digestion with DNase I. Polylysine was then removed by dissociating the polylysine-DNA complex in 2M NaCl and separating the mixture of DNA and polylysine on a column of hydroxyapatite (HAP). The validity of the isolation procedure and the physiochemical properties of the DNA preparation are reported elsewhere (14). The DNA was further treated to remove a small fraction (~ 15%) of single-stranded DNA routinely found in accessible DNA preparations. The DNA was applied to an HAP column held at 60° and equilibrated in 0.12M Na phosphate, pH 6.8. The DNA which passed through the column (single-stranded DNA) was discarded. The absorbed DNA (double-stranded fraction), representing 85-90% of the applied DNA, was eluted with 0.4M Na phosphate and precipitated as the cetyltrimethylammonium bromide (CTAB) salt. After reconversion to the sodium salt the DNA was dissolved in 0.12M Na phosphate, pH 6.8.

Isolation of Whole Chromatin DNA: Whole chromatin DNA was isolated from chromatin as described previously (14). The purified DNA was extensively sonicated to reduce the fragment length to the same size as that of the accessible DNA preparation (200-250 base pairs).

Preparation of the Nonrepeated Fraction of Accessible DNA and Whole Chromatin DNA: Solutions of accessible DNA or whole chromatin DNA at a concentration of approximately 1 mg/ml in 0.12M Na phosphate buffer, pH 6.8, were denatured in a boiling water bath for 10 min and allowed to reassociate at 60° until a C₀t of 250 had been reached. The DNA was then fractionated into single and double-stranded molecules on a HAP column (0.12M Na phosphate buffer, 60°). The single-stranded (nonrepeated)

fraction eluting at 0.12M Na phosphate was collected and passed through a second HAP column in 0.12M Na phosphate at 60° to remove any contaminating double-stranded (repetitive) DNA that may not have bound to the first column. The DNA which passed through the second column was collected, precipitated with CTAB and taken up as the sodium salt in a small volume of 0.12M Na phosphate buffer. For both DNA samples approximately 60% of the input DNA was recovered in the nonrepeated DNA fraction.

In vitro Labeling of DNA with ^{125}I : Nonrepeated DNA fractions were labeled in vitro with ^{125}I essentially by the procedure of Commerford (15). The iodinated DNA's were further purified by HAP chromatography and CTAB precipitation. The DNA was applied to a 60° HAP column in 0.03M Na phosphate, washed with 0.03M Na phosphate, and eluted with 0.40M Na phosphate. The DNA was then precipitated with CTAB and redissolved as the Na salt as described previously. The specific activities of labeled DNA's used in these experiments ranged from 5×10^5 to 1.1×10^6 cpm/ μg .

Determination of Reassociation Kinetics: Reassociation kinetics were measured by the HAP method of Britten and Kohne (16). DNA samples were dissociated in a boiling water bath and incubated in either 0.12M Na phosphate, pH 6.8, at 60° or 0.48M Na phosphate at 68°. After adjustment of the salt concentration to 0.12M where necessary, the samples were applied to one ml columns of HAP maintained at 60°. Single-stranded DNA was eluted with 0.12M Na phosphate and double-stranded (reassociated) DNA with 0.40M Na phosphate.

Isolation of Total Nuclear RNA: Nuclear RNA was isolated and purified from rat liver nuclei by the hot phenol-SDS method. The procedure used was essentially that described by Church (17). The purification process included treating the RNA twice with pancreatic DNase to remove contaminating DNA, passing the RNA through a Sephadex G-50 column to remove degraded RNA, DNA, and other low molecular weight compounds, and precipitating the RNA with CTAB in the presence of EDTA to remove neutral polysaccharides and metal ions. The RNA was also sheared to increase hybridization efficiency (17). The shearing was accomplished by sonicating the RNA (2 mg/ml in 0.1M NaCl, 0.01M tris-HCl, pH 8.0, 0.001M EDTA) for a total time of 2 min in a Branson S125 Sonifier at 20 kHz with a power setting of 96W. After concentration of the RNA it was dissolved in 0.5M Na phosphate buffer, pH 6.8 and stored frozen.

RNA-DNA Hybridization: DNA samples were hybridized with nuclear RNA until saturation was reached to determine what percentage of the DNA is complementary to sequences present in the RNA population (18,17). A small

quantity of ^{125}I -labeled DNA was mixed with a vast excess of nuclear RNA and hybridized for various times. Each reaction mixture contained 10.5mg RNA/ml and 2.05 μg nonrepeated ^{125}I -DNA/ml (RNA:DNA ratio of 5000 to 1) in 0.36M Na phosphate buffer, pH 6.8, 0.001M EDTA. Final volume was 0.075 ml. The reaction mixtures were dissociated at 104° for 7 min and incubated in sealed tubes at 70° for the desired times. The tubes were then diluted and the amount of RNA-DNA hybrid formed assayed either with HAP or with S_1 nuclease as described below.

Assay of Hybrids with HAP: RNA-DNA hybridization reaction mixtures were diluted with 2.3 mls of 0.12M Na phosphate, pH 6.8, and one ml aliquots were applied to one ml columns of HAP equilibrated in 0.12M Na phosphate at 60° . After extensive washing of the column with 0.12M Na phosphate, RNA-DNA hybrids were eluted with 0.4M Na phosphate.

Assay of Hybrids with S_1 Nuclease: Hybrid formation was assayed with S_1 nuclease essentially by the method of Sutton (19). RNA-DNA hybridization reaction mixtures were diluted with 2.3 mls of nuclease buffer (0.04M Na acetate, pH 4.5, 0.01M NaCl, $1 \times 10^{-4}\text{M}$ ZnSO_4). 4000 (Miles) units of S_1 nuclease were added and the samples were incubated for 30 min at 50° . After incubation undigested DNA (present in hybrids) was precipitated by making the solution 5% in trichloroacetic acid (TCA) and 0.1% in bovine serum albumin. The precipitated hybrid was washed twice in 5% TCA and then dissolved in 1N NaOH.

RESULTS

Characterization of the Nonrepeated (Unique Sequence) Fraction of Accessible and Whole Chromatin DNA: In order to remove repeated DNA sequences which would interfere in the RNA-DNA hybridization studies, both accessible and whole chromatin DNA were dissociated and reassociated to a C_0t value sufficient to allow most of the repeated sequences to reassociate but not to allow the bulk of the nonrepeated sequences to form hybrids. The reassociated sequences were then removed by binding to HAP. By analysis of the reassociation profile of accessible and whole chromatin DNA (given in Ref. 14) a C_0t of 250 was chosen for this separation. This value has also been frequently used by other workers (17) to isolate the nonrepeated fraction of animal DNA's.

Fig. 1 shows the reassociation profile of the isolated nonrepeated DNA fractions. At the highest C_0t attempted ($\text{C}_0\text{t} = 12,000$) the reaction goes to 85% completion. At this point 98% of the DNA is still acid insoluble. The shape of the curve indicates the reaction approximates a

Fig. 1: Reassociation profile of nonrepeated fractions of accessible and whole chromatin DNA. Reassociation of DNA samples was measured on hydroxyapatite columns as described in text. —●— accessible DNA, —○— whole chromatin DNA.

true second order rate process which one would expect of a reassociating DNA containing only unique sequences. Good base pairing fidelity of the reassociated hybrids was indicated by only a small decrease in the T_m of the hybrids as measured by thermal chromatography of the hybrids directly from the column. The T_m of the accessible DNA hybrid (formed at a C_0t of 10,000) was 87° and the total DNA hybrid, 86° . The T_m of native DNA was 90° .

Within experimental error the two curves are identical with a $C_0t_{1/2}$ of 1.7×10^3 . Assuming the base composition and fragment length of the two DNA samples are the same this unexpected result implies that the sequence complexity of the two DNA's is the same or very similar. Even if we assume a small difference in the base composition of the two DNA's as is found between the unfractionated DNA's (14) the correction will not change the $C_0t_{1/2}$ value to any great degree; that is, if we assume that accessible nonrepeated DNA is 38% G-C and total nonrepeated DNA is 41% as is found for the unfractionated DNA's, then the reassociation of accessible DNA will be retarded only slightly. Using the data of Wetmur and Davidson (20) the rate of its reassociation will be reduced by a factor of 0.93.

When this correction is applied and assuming that the observed $C_{ot1/2}$ values could vary between 1.5 and 1.9×10^3 due to experimental error, the maximum amount of sequence difference could only be 15%. That is, accessible DNA must contain at least 85% of all the nonrepeated sequences present in total DNA.

Analysis of Sequence Homology by Reassociation of Total DNA with a Vast Excess of Accessible DNA: To verify the finding of sequence similarity by an alternate method, we performed a driver-trace reassociation experiment. A small quantity of ^{125}I -labeled nonrepeated whole chromatin DNA was mixed with a large amount of unlabeled accessible DNA. The mixture was dissociated and the course of reassociation followed both optically and by radioactivity. The concentration of labeled total chromatin DNA was made low enough so that self-reassociation of labeled strands would be insignificant during the course of the reaction and the rate of reassociation of these strands would be governed almost entirely by the concentration of the complementary unlabeled sequences present in the accessible DNA driver. The rationale behind this experiment is that if "accessible" DNA contains only a specific fraction of all sequences present in whole chromatin DNA, then total DNA will contain a set of sequences not present in accessible DNA. During a driver-trace reassociation reaction these additional sequences present in total DNA will not be able to find complementary sequences in accessible DNA and therefore a trace amount of total DNA can not completely reassociate in the presence of an accessible DNA driver. Thus the trace total DNA should appear to reassociate at a slower rate than the accessible DNA driver. Before this experiment was attempted, however, an anomalous labeling effect was discovered. It was found that even if the trace and driver DNA's were the same (that is, if labeled accessible DNA were reassociated in the presence of unlabeled accessible DNA or labeled total DNA in the presence of unlabeled total DNA) the reassociation of the labeled DNA (as measured by radioactivity) was much slower than the driver DNA (as measured by optical absorbance). At a C_{ot} of 12,000, 85% of the unlabeled DNA but only 65% of the labeled DNA will reassociate. Thus the labeling of the DNA has somehow altered its apparent rate of reassociation. There are two possible reasons for this effect: 1) the labeling procedure itself could cause fragmentation of the DNA resulting in a reduced fragment size and 2) the chemically altered DNA might not form hybrids as well as unlabeled DNA possibly on account of the bulky iodine molecule present on the cytosine bases. The fragmentation of DNA by iodination has been noted by

other workers (21) and we have confirmed this ourselves by observing that iodinated DNA has a lower molecular weight (as judged by sedimentation in an alkaline sucrose gradient) than the original DNA. To eliminate this inherent problem from the driver-trace reassociation experiment, a second set of reassociation mixtures were run at the same time. These control mixtures contained trace amounts of labeled accessible DNA in the presence of unlabeled accessible DNA.

The results of the experiment are given in Fig. 2. The reassociation of the accessible DNA driver which is not shown was the same as shown in Fig. 1. Because the two curves are very similar and total DNA reassociates only slightly slower than accessible DNA, the same conclusion is reached as before; that is, accessible DNA has at least 85% of the unique sequences present in total DNA.

Hybridization of Nonrepeated DNA's to Liver Nuclear RNA: Although nonrepeated accessible DNA seems to have, by DNA-DNA reassociation experiments, very nearly the same sequence composition as whole chromatin DNA,

Fig. 2: Reassociation profile of trace amounts of ^{125}I -labeled non-repeated accessible and nonrepeated whole chromatin DNA incubated in the presence of a vast excess of unlabeled nonrepeated accessible DNA. Unlabeled DNA to labeled DNA ratio: 500:1. Reassociation of samples measured on hydroxyapatite columns. —●— accessible DNA, —○— whole chromatin DNA.

we hybridized the DNA's against liver nuclear RNA to see if accessible DNA could still be enriched in sequences transcribed in vivo. Each DNA was labeled in vitro and hybridized against a vast excess of liver nuclear RNA. The hybridization reaction was continued until no more RNA would hybridize with the DNA.

In one experiment the formation of hybrids was measured on hydroxyapatite. The results are shown in Fig. 3. The saturation value for accessible DNA was 4.9% and that for total DNA 5.3%. This difference may not be significant as the error on each hydroxyapatite assay is about $\pm 0.5\%$. Although hydroxyapatite will not distinguish between RNA-DNA hybrids and DNA-DNA hybrids in this assay, we estimate that at least 80% of the binding DNA is in the form of RNA-DNA hybrids. No more than 20% of the DNA is present in DNA-DNA hybrids because of the following data. First, the concentration of the labeled DNA was made extremely dilute so that even after 9 days of incubation the DNA C_0t would only reach a value of 2. From the reassociation curve of the nonrepeated DNA's only 1-2% of the DNA could be expected to self-reassociate at this C_0t . This fact was determined experimentally by incubating DNA alone (in the presence of E. coli carrier DNA) and measuring the hybrid formation after 5 days of incubation. Only 1.5% of the radioactivity appeared in the hybrid fraction. Secondly, any DNA-DNA hybrids resulting from either self-reassociation of the labeled DNA or from hybridization of the labeled DNA with any contaminating DNA present in the nuclear RNA preparation were determined by assaying hybrid formation after labeled DNA was incubated with nuclear RNA which had been predigested with pancreatic RNase. In this case no RNA-DNA hybrids could form and observed hybrids would have to arise from DNA-DNA hybridization. The results are shown in Fig. 3 and indicate that even after long incubation only about 1.5% of the labeled DNA formed hybrids.

In another experiment the hybridization reactions were run in an identical manner but the formation of hybrids was measured with the use of S_1 nuclease. The results are shown in Fig. 4. The saturation value for accessible DNA is 7.8% and for whole chromatin DNA, 9.0%. The difference in these values could be significant as the error of each nuclease assay was calculated as $\pm 1\%$. In this experiment an additional control was run to determine nonspecific binding of DNA (over and above 0 time background levels). For this determination a non-homologous RNA (wheat germ ribosomal RNA) which should have few complementary sequences with nuclear RNA was used. Hybridization reaction mixtures were set up as usual but an equivalent amount of wheat germ RNA replaced the nuclear RNA. The results

Fig. 3: Hybridization of nonrepeated accessible and nonrepeated whole chromatin DNA to a vast excess of sheared liver nuclear RNA. RNA to DNA ratio 5000:1. Reaction conditions given in Methods. Hybrid formation assayed on hydroxyapatite columns as described in text. Each point represents the average of at least two independent determinations. —●— accessible DNA, —○— whole chromatin DNA, ---●--- accessible DNA hybridized to nuclear RNA predigested with RNase, ---○--- whole chromatin DNA hybridized to nuclear RNA predigested with RNase.

are also shown in Fig. 4. No more than 1% of the DNA appeared in hybrids at the latter stages of the reaction.

The reason for the discrepancy between the hydroxyapatite and nuclease determined saturation values is not known. The most probable explanation is that suggested by Leong *et al.* (22) who compared HAP and nuclease methods in RNA-DNA hybridizations. They found that HAP fails to bind very low molecular weight RNA-DNA hybrids and therefore the HAP method underestimates hybrid formation especially when low molecular weight DNA is being used. Our starting DNA preparations are only 200–250 nucleotides long and these are then subjected to a lengthy incubation procedure to isolate the nonrepeated fraction and are then unavoidably fragmented further during the iodination procedure.

Although the hybridization curves level off in both experiments we

Fig. 4: Hybridization of nonrepeated accessible and nonrepeated whole chromatin DNA to a vast excess of sheared liver nuclear RNA. Reaction conditions same as those in Fig. 3 but hybrid formation assayed with S_1 nuclease. Each point represents the average of at least two independent determinations. Background (0 time) binding of 2.3% has been subtracted from all values. —●— accessible DNA, —○— whole chromatin DNA, ---■--- whole chromatin DNA hybridized to nuclear RNA predigested with RNase, ...x... whole chromatin DNA hybridized to wheat germ ribosomal RNA.

can not be sure that full saturation of the DNA's has been reached. The stability of the RNA during the course of the reaction will obviously affect the observed saturation values. Our RNA preparation though relatively stable during the hybridization did exhibit some signs of degradation after long incubations. After 9 days, 10% of the RNA (as well as 125 I-DNA counts) had become acid soluble. The slow degradation of RNA was also evidenced by the observation that less RNA bound to HAP as the hybridization progressed indicating a destruction of secondary structure. Of more theoretical importance is the question of whether the duration of the reaction is sufficient to allow those RNA sequences which are most dilute in the nuclear RNA preparation to hybridize. Present methods provide no assurance that all RNA sequences complementary to DNA can hybridize regardless of the number of copies present.

In summary, the RNA-DNA hybridization results show that accessible DNA and whole chromatin DNA hybridize to liver RNA to about the same extent.

The slight reduction in the hybridization of accessible DNA compared to that of whole DNA may be significant, however. These results indicate, in effect, that accessible DNA has the same amount of (or perhaps slightly fewer) transcribed sequences as whole chromatin DNA.

DISCUSSION

The data presented here indicate that nonrepeated accessible DNA, isolated by polylysine-binding, is not preferentially transcribed in the cell. If accessible DNA represents only 50% of the genome but contains all genes transcribed in vivo then we would expect it to hybridize with twice the amount of nuclear RNA that will hybridize with whole chromatin DNA. The RNA-DNA hybridization data, however, show no increase in hybridizability and in fact indicate a possible decrease for this DNA fraction. This finding would imply that all structural genes whether transcribed or not are equally accessible in chromatin. This is not to say that chromosomal protein-DNA interactions play no part in transcriptional control. The control of transcription could reside in only a few proteins located (or absent) at only the operator region of each gene. This type of control would probably not be detectable by polylysine accessibility studies.

These RNA-DNA hybridization results are completely compatible with the recently published findings of Axel, Cedar, and Felsenfeld (23). Using a probe for the globin gene, they studied accessible DNA as well as protein-associated ("covered") DNA in reticulocyte chromatin. Their accessible DNA was also prepared by polylysine-binding. By hybridization of accessible and covered DNA against the DNA complementary to globin mRNA, they found that not only does accessible DNA contain most of the globin gene but also that the gene is present in similar amounts in covered DNA. They deduced from this finding that transcribed structural genes do not have to be accessible to be transcribed. It is interesting to note also that they found a small percentage of the globin gene sequence was missing from accessible DNA while we find that there is possibly a small reduction in binding of nuclear RNA to this fraction.

As we have performed these studies only with non-repeated DNA we can say little about the transcription of the repeated fraction of accessible DNA although the non-repeated fraction should contain at least one copy of each repeated DNA family. We have not studied the transcription of the repeated DNA fraction because of the inherent ambiguities involved in interpreting the results of hybridization experiments between repeated DNA

and nuclear RNA. A specific copy of a repeated DNA sequence could be repressed in accessible DNA while another copy of the same sequence located elsewhere in protein-covered DNA could be expressed. The RNA transcript of this sequence would, however, hybridize equally well to both sequences in deproteinized DNA. It should be noted here that the studies of Axel *et al.* (23) were made with accessible DNA containing all repetitive and unique sequences.

Our results as well as those of Axel *et al.* seem to conflict with earlier findings (9,10,11) that "template-active" fractions of chromatin bind more polylysine than "template-inactive" fractions or unfractionated chromatin. The hybridization data, however, only indicate that the increased binding of polylysine to "template-active" chromatin is not related to an increase of transcribed sites. The increased binding must, therefore, reside in some other property of this chromatin fraction. For instance, some polylysine may bind to acidic proteins (which are found in high concentrations in "template-active" fractions) or more polylysine may bind to DNA partially covered with histone because the electrostatic histone-DNA interaction could be weakened by the presence of acidic proteins. There is also the possibility that binding of polylysine to these chromatin fractions could be increased due to some alteration in chromatin structure created by the fractionation process itself. For instance, the "template-active" fractions prepared in our own laboratory are obtained by solubility behavior in dilute Mg^{++} solutions. In this instance alteration of chromatin structure may be due to interactions of the Mg^{++} with DNA phosphates or chromatin proteins.

The experiments reported here also show that accessible DNA has almost all of the unique sequences present in the genome. Similar results were obtained by Axel *et al.* (23) with reticulocyte chromatin. The most straightforward explanation of this finding is that accessible DNA sites are located randomly in chromatin. This randomness could reflect either the true state of chromatin structure in the cell or result from the migration of protein during chromatin isolation. Axel *et al.* have argued for the first explanation and have postulated that the arrangement of histone on a particular DNA fiber is essentially random. Many investigators have shown previously that, at least *in vitro*, histones do not seem to bind to specific DNA sequences (24,25).

Recently a new model of chromatin structure has been emerging in which chromatin is seen as being constructed of repeating subunits (see Ref. 26 for review). Each subunit consists of a short length of DNA

complexed with a cluster of interacting histone molecules to form a "bead." The beads are then spaced along the DNA fiber separated by a short piece of relatively protein-free DNA. When this model was first proposed it was suggested that the spacer DNA might be the accessible DNA found in chromatin. But later discoveries suggest that the DNA in the bead might also be relatively accessible. Baldwin *et al.* (27) using neutron diffraction and others (28) suggest that the DNA in the bead might wind around the protein complex and thus put the DNA on the outside of a protein core. Therefore, this DNA although in close association with protein could still be partly accessible to chemical probes. There is some evidence obtained by melting and DNase susceptibility studies that polylysine can bind to histone-covered DNA (5,29).

Our data as well as that of Axel *et al.* (23) suggest another aspect of this model. To be consistent with our results the beads must be located randomly on the DNA fiber (although still separated from one another) and not be confined to specific nucleotide sequences, or, alternatively, the beads must be free to slide along the DNA.

A closing precautionary statement should be made at this time. All of our results as well as those of Axel *et al.* (23) are based on the assumption that accessible DNA is real and can be isolated by polylysine binding. In a previous paper (14) we summarized the reasons for believing this assumption to be reasonable. If, however, polylysine can migrate from its original binding sites to different DNA molecules during the isolation procedure, it is possible that an essentially random population of DNA sequences could be produced and this process would lead to exactly the results that we and Axel *et al.* find. Because of the close packing of DNA in chromatin the possibility exists that polylysine could bind to a protein-associated portion of DNA in which the DNA phosphates are not totally neutralized (or bind directly to protein) and during pronase digestion when free DNA is produced, "jump" from its weakly bound site to an adjacent segment of free DNA. Recently Oliver and Chalkley (30) have discussed the possibility of artifactual production of nucleoprotein particles during DNase digestion of chromatin by a similar process, although they were concerned only with the migration of histone molecules. Because of this possibility the actual mechanism of polylysine binding to chromatin needs further investigation.

It should also be noted that we have used sheared chromatin in these experiments and a recent paper (31) presents evidence that shearing may alter the native structure of chromatin. However, we believe that our

chromatin preparations are not significantly altered for the following reasons: 1) the conditions of shear reported by Noll et al. (31) are much more severe than the 8 second sonication that we utilize; 2) other reports (32,33) utilizing extensive shearing conditions demonstrate by a variety of procedures that changes in chromatin structure are minimal; 3) earlier studies reported from our laboratory (9) indicate that isolated chromatin subjected to an 8 second sonication retains many features of non-sheared chromatin and has a molecular weight $> 15 \times 10^6$ daltons. The final resolution of this point is dependent upon future studies on accessibility to chemical probes in native chromatin.

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REFERENCES

- 1 Clark, R.J. and Felsenfeld, G. (1971) *Nature*, New Biol. 229, 101-106.
- 2 Itzhaki, R.F. (1971) *Biochem. J.* 122, 583-592.
- 3 Axel, R., Cedar, H. and Felsenfeld, G. (1974) in *Cold Spring Harbor Symposia on Quantitative Biology*, Vol. XXXVII, pp 773-783, Cold Spring Harbor Laboratory.
- 4 Itzhaki, R.F. (1974) *European J. Biochem.* 47, 27-33.
- 5 Li, J.H., Chang, C. and Weiskopf, M. (1972) *Biochem. Biophys. Res. Commun.* 47, 883-887.
- 6 Varshavsky, A.J., Ilyin, Y.V. and Georgiev, G.P. (1974) *Nature* 250, 602-606.
- 7 Simpson, R.T. (1970) *Biochemistry* 9, 4814-4819.
- 8 Simpson, R.T. (1973) in *Advances in Enzymology*, Vol. 38, pp 41-108, John Wiley, New York.
- 9 Arnold, E.A. and Young, K.E. (1974) *Arch. Biochem. Biophys.* 164, 73-89.
- 10 Simpson, R.T. and Polacow, I. (1973) *Biochem. Biophys. Res. Commun.* 55, 1078-1084.
- 11 Marfey, S.P. and Li, M.G. (1974) *Nature* 249, 559-560.
- 12 Grouse, L., Chilton, M.D. and McCarthy, B.J. (1972) *Biochemistry* 11, 798-805.
- 13 Itzhaki, R.F. and Cooper, H.K. (1973) *J. Mol. Biol.* 75, 119-128.
- 14 Arnold, E.A., Wahn, U. and Young, K.E. (1975) *Nucleic Acids Res.* 2, 667-681.
- 15 Commerford, S.L. (1971) *Biochemistry* 10, 1993-1999.
- 16 Britten, R.J. and Kohne, D.E. (1968) *Science* 161, 529-540.
- 17 Church, R.B. (1973) in *Molecular Techniques and Approaches in Developmental Biology*, pp 223-301, John Wiley, New York.

- 18 Kohne, D.E. (1968) *Biophys. J.* 8, 1104-1118.
- 19 Sutton, W.D. (1971) *Biochim. Biophys. Acta* 240, 522-531.
- 20 Wetmur, J.G. and Davidson, N. (1968) *J. Mol. Biol.* 31, 349-370.
- 21 Orosz, J.M. and Wetmur, J.G. (1974) *Biochemistry* 13, 5467-5473.
- 22 Leong, J., Garapin, A.C., Jackson, N., Fanshier, L., Levinson, W. and Bishop, J.M. (1972) *J. Virol.* 9, 891-902.
- 23 Axel, R., Cedar, H. and Felsenfeld, G. (1975) *Biochemistry* 14, 2489-2495.
- 24 Baldwin, J.P., Bradbury, E.M., Butler-Browne, G.S. and Stephans, R.M. (1973) *FEBS Letters* 34, 133-136.
- 25 Zimmerman, S.B. and Levin, C.J. (1975) *Biochem. Biophys. Res. Commun.* 62, 357-361.
- 26 Elgin, S.C.R. and Weintraub, H. (1975) in *Annual Reviews of Biochemistry*, Vol. 44, pp 725-774, Annual Reviews Inc., Palo Alto, California.
- 27 Baldwin, J.P., Boseley, P.G., Bradbury, E.M. and Ibel, K. (1975) *Nature* 253, 245-249.
- 28 Hyde, J.E. and Walker, I.O. (1975) *Nucleic Acids Res.* 2, 405-421.
- 29 Billings, R.J. and Bonner, J. (1972) *Biochim. Biophys. Acta* 281, 453-462.
- 30 Oliver, D. and Chalkley, R. (1974) *Biochemistry* 13, 5093-5098.
- 31 Noll, M., Thomas, J.O. and Kornberg, R.D. (1975) *Science* 187, 1203-1206.
- 32 Reeck, G.R., Simpson, R.T. and Sober, H.A. (1972) *Proc. Nat. Acad. Sc. U.S.A.* 69, 2317-2321.
- 33 Doenecke, D. and McCarthy, B.J. (1975) *Biochemistry* 14, 1373-1378.