Distribution of Tightly Bound Proteins in the Chicken Ovalbumin Gene Region†

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ABSTRACT: A method for investigating the distribution of the DNA–tightly bound proteins (TBP) in the chicken ovalbumin gene regions is described. The TBP are operationally defined as the proteins that remain bound to the DNA in the presence of 2 M NaCl. Nuclei from chicken erythrocytes, livers, and oviducts were lysed in 2 M NaCl and sheared, and the lysed chromatin was chromatographed through a Sepharose 4B column to separate protein and DNA which contains the TBP. The DNA fraction, after digestion with restriction endonuclease *Eco*RI, was passed through a GF/C glass fiber filter. The filter retains the DNA–TBP but not the protein-free DNA. In all cases, only about 0.5% of the total genomic DNA was retained on the filter. The ovalbumin gene sequences in the DNA–TBP were analyzed by the Southern blot. It was found that the copy number of the ovalbumin gene in the DNA–TBP isolated from erythrocyte or liver nuclei is not significantly different from that in the total unfractionated nuclear DNA. Liquid hybridization of nick-translation-labeled DNA–TBP with a large excess of total chicken nuclear DNA also demonstrated that the DNA is not a specific subset of the genome. In the nuclei from laying hen oviducts in which the ovalbumin gene but not the globin is actively expressed, approximately 3-fold enrichment of the ovalbumin gene was found in the DNA–TBP. The enrichment could have been due to a contamination of transcriptional complexes during the purification of the DNA–TBP, since no depletion in the globin gene sequences was found in the same sample. These results suggest a random distribution of the TBP in that genome, due possibly to a transient interaction between DNA and TBP. Precautions for evaluation of the results dealing with the TBP published in the literature are also discussed.

It has been known for some time that high molar salt treatment of the cell nucleus removes most of the nuclear proteins (Mirsky & Ris, 1951; Berezney & Coffey, 1975; Comings, 1978). The residual chromosomal proteins, which are essentially nonhistone proteins and presumably tightly bound to the DNA, have been proposed to be nuclear matrix proteins in interphase nuclei (Berezney & Coffey, 1975; Comings, 1978; Long et al., 1979) and scaffold proteins in metaphase chromosomes (Laemmli et al., 1977). The nuclear matrix and chromosome scaffold have been thought to play an important role in maintaining the highly ordered structure of chromatin/chromosomes, so that DNA replication, gene expression, and other cellular functions are correctly regulated (Long et al., 1979; Pardoll et al., 1980). Recent reports also suggest that the tightly bound proteins (TBP) are tissue specific and may be related to regulatory elements of gene expression (Gates & Bekhor, 1979a,b; Bekhor & Mirell, 1979; Pumo et al., 1980; Norman & Bekhor, 1981).

At present, very little information is available concerning the nature of the TBP and their interactions with DNA. Knowing the DNA sequences associated with the TBP is particularly important in order to formulate the mode of interactions between DNA and TBP and to gain new insights into the functional role of TBP in cellular physiology. The present report was initiated to investigate whether there are any specific DNA sequences associated with the TBP in the chicken ovalbumin gene region. The results thus far obtained show that the distribution of the TBP is not particularly DNA sequence specific.

Materials and Methods

Preparation of Nuclei and Chromosomes. Nuclei from chicken erythrocytes were isolated according to the described procedure (Wilhelm & Champagne, 1969). From laying hen oviducts or livers, nuclei were isolated by the procedure of Hewish & Burgoyne (1973). The detailed procedure for isolation of chromosomes and nuclei from chicken lymphoblastoid cell MSB-1 will be published elsewhere (Kuo, 1982).

Column Chromatography of Sheared Chromatin. All procedures were performed at 4 °C with siliconized glassware or plasticware unless otherwise indicated. The nuclei were suspended in a solution containing 15 mM Tris-HCl, pH 7.4, 60 mM KCl, 15 mM NaCl, 15 mM β-mercaptoethanol, 0.15 mM spermine, 0.5 mM spermidine, and 0.34 M sucrose at a 260-nm absorbance of 24 (measured in 2 M NaCl). To 3 mL of nuclear suspension was added dropwise 2 mL of 5 M NaCl with constant vortexing. The nuclear lysate was sheared by passing it through a 20-gauge needle 15 times. The molecular weight of DNA isolated from the sheared sample was greater than 35 kilobases (kb) as analyzed by 0.4% agarose gel electrophoresis using intact adenovirus 2 DNA as a marker.

The sheared sample was stirred for 30 min and then chromatographed through a Sepharose Cl-4B (Pharmacia) column (2.5 × 60 cm) which had been equilibrated with a solution containing 2 M NaCl, 10 mM Tris-HCl, pH 7.9, and 1 mM ethylenediaminetetraacetic acid (EDTA) and eluted with the same solution. Absorbances at 230 and 260 nm in each fraction (5 mL) were measured.

Restriction Enzyme Digestion of DNA. The DNA fractions from the Sepharose column chromatography were pooled and concentrated by centrifugation onto a 1-mL sucrose cushion containing 2.4 M sucrose, 10 mM Tris-HCl, pH 7.9, and 1 mM EDTA in a Beckman SW 41 rotor at 40 000 rpm for 7

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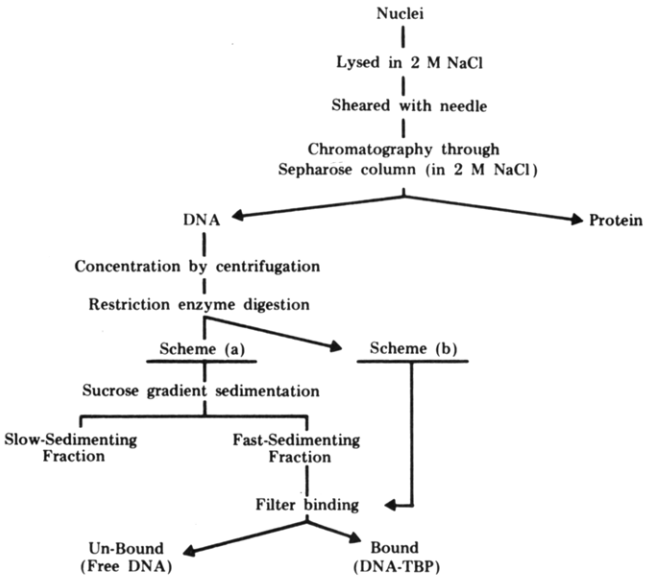

FIGURE 1: Schematic diagram showing the procedure for the preparation of DNA–tightly bound proteins.

h. After it was centrifuged, the DNA was dialyzed overnight against a buffer containing 10 mM Tris-HCl, pH 7.4, 50 mM NaCl, and 1 mM EDTA. The DNA was digested to completion with restriction enzyme *Eco*RI in the presence of 10 mM $MgCl_2$.

Sucrose Gradient Sedimentation and Filter Binding. The *Eco*RI-digested DNA was layered onto a sucrose step gradient comprised of the following (from the bottom): 4 mL of 1.2 M sucrose containing 10 mM Tris-HCl, and 1 mM EDTA, 4 mL of 2 M NaCl, and 3 mL of the sample. The gradients were spun in a Beckman SW 41 rotor at 40 000 rpm for 6 h and fractionated form the top (1 mL/fraction). Absorbances at 260 and 280 nm in each fraction were measured. The bottom three fractions from each gradient, designated as fast-sedimenting fractions, were pooled and dialyzed against a buffer containing 10 mM Tris-HCl, pH 7.9, 1 mM EDTA, and 0.2 M NaCl (binding buffer).

Filter binding of the DNA–protein complex in the restriction enzyme digested sample was performed essentially according to the described procedure (Coombs & Pearson, 1978) with slight modification. The dialyzed sample from the sucrose step gradient (2000 μg of DNA/5 mL) was passed through a 2.4-cm Whatman GF/C glass fiber filter for 2 or 3 times using mild suction. The filter, which had been presoaked in the binding buffer, was installed in a Millipore filtration assembly with suctioning. The filter was then rinsed with 5 mL of binding buffer and transferred into an Eppendorf tube that contained 1.0 mL of eluting buffer [10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 0.1% sodium dodecyl sulfate ($NaDodSO_4$)]. The filter was treated with RNase A (50 μg/mL) followed by proteinase K (50 μg/mL), each for 60 min at 37 °C. A hole was punched at the bottom of the Eppendorf tube, and the eluting buffer was centrifuged into a glass tube (12 × 75 mm) in a Sorvall centrifuge. The DNA was extracted from the eluent with phenol–chloroform–isoamyl alcohol (24:24:1 v/v) and precipitated with ethanol. The concentration of DNA was determined by a fluorometric method (Hinegardner, 1971).

Other Procedures. Analytical procedures including preparation of DNA probes, labeling of DNA by nick translation (Rigby et al., 1977), Southern blotting–hybridization (Southern, 1975), $NaDodSO_4$–polyacrylamide gel electrophoresis of proteins (Laemmli, 1970), and liquid hybridization of labeled DNA with sonicated chicken nuclear DNA (Kuo et al., 1976) have been described previously.

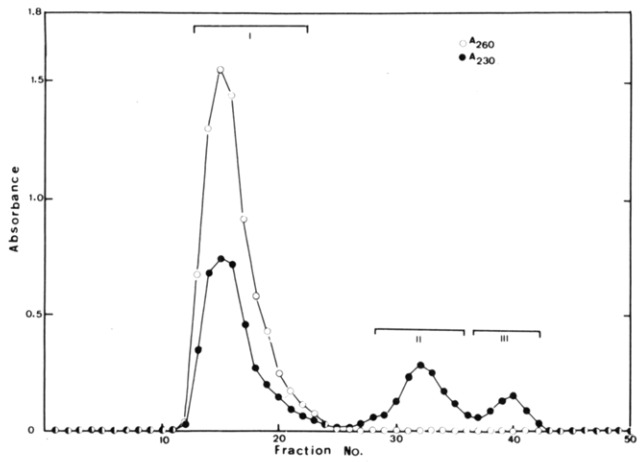

FIGURE 2: Sepharose 4B chromatographic separation of DNA and protein in chicken erythrocyte chromatin.

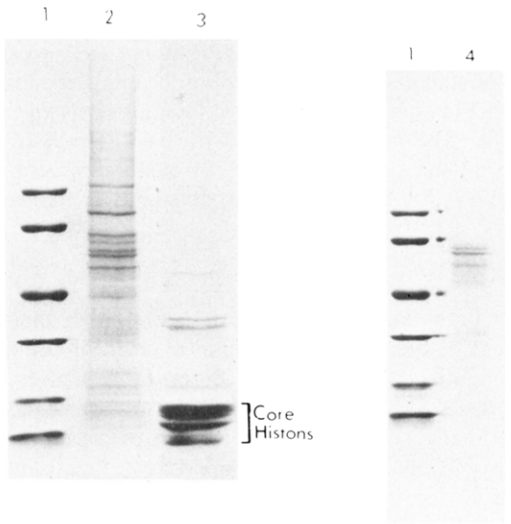

FIGURE 3: $NaDodSO_4$–polyacrylamide gel electrophoresis of proteins in the fractions eluted from the Sepharose 4B columns: Lane 1, protein standards (from the top, $\times 10^3$ daltons) are phosphorylase *b* (94), bovine serum albumin (67), ovalbumin (43), carbonic anhydrase (30), soybean trypsin inhibitor (20), and α-lactalbumin (14); lane 2, sample from peak I; lane 3, sample from peak II; lane 4, sample from peak I. Samples in lanes 3 and 4 were obtained from chicken erythrocyte nuclei. Sample in lane 2 was from chicken oviduct nuclei. Peaks I and II are shown in Figure 2.

Results

Preparation of DNA–Tightly Bound Protein Complexes. A schematic diagram of the procedure for the preparation of the DNA–tightly bound proteins (DNA–TBP) is shown in Figure 1. The nuclei isolated from chicken erythrocytes, livers, and oviducts were lysed in 2 M NaCl, sheared, and chromatographed through a Sepharose 4B column. The protein and DNA were efficiently separated as monitored by the absorbances of wavelengths at 230 and 260 nm, respectively (Figure 2). The DNA was eluted as a single peak (fractions 13–24, peak I), whereas proteins were found in two peaks (fractions 28–36, peak II, and 37–42, peak III). I have checked this point by using the sample isolated from chicken MSB-1 cells which had been labeled with [^{14}C]thymidine and [^{3}H]lysine. Using the labeled sample, it was also found that virtually no DNA was cochromatographed with the protein fractions, and very

little of the DNA was retained in the column (>98% recovery).

The proteins in peaks I, II, and III were precipitated with ethanol and analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. Both peaks II (Figure 3, lane 3) and III (not shown) contained predominantly histones. In agreement with results published in a previous report (Eickbush & Moudrianakis, 1978), peak II contained the octomeric core histones, while peak III contained the dissociated core histones.

Several major species of proteins with molecular weights ranging from 45 000 to 67 000 were found to associate with the DNA-containing fractions, when chicken erythrocyte nuclei were used as starting material (Figure 3, lane 4). Proteins associated with the DNA fractions from chicken oviduct nuclei that had undergone the same procedure were different from those prepared from chicken erythrocyte nuclei (Figure 3, lane 2). More species of proteins, including both high and low molecular weight molecules, were seen in the samples isolated from oviduct nuclei. No detectable histones, however, were found in the DNA fractions (Figure 3, lanes 2 and 4). It is not the major purpose of this study to characterize the differences in DNA-bound proteins in the nuclei isolated from different sources. This is because the proteins shown in the gel are not certain to be associated with DNA during preparation. Some proteins may be cochromatographed with DNA. It is also not proved that all the proteins found in the DNA fractions are indeed of nuclear origin. Some proteins may be cytoplasmic contaminants that appear during preparation of nuclei.

The DNA fractions were pooled and concentrated by sedimentation onto a sucrose cushion. The DNA recovered from the sucrose cushion (95% recovery) was digested with the restriction endonuclease *Eco*RI. Subsequently, two methods were used to isolate the restriction genomic DNA containing the TBP (see Figure 1). Method a involves a sucrose step gradient sedimentation and filter binding, while method b consists of only the filter binding. In method a, the *Eco*RI-digested DNA samples were sedimented through a sucrose gradient as described under Materials and Methods. The bottom three fractions of the gradient, about 10% of the total input DNA, were designated as fast-sedimenting fractions. It was found that these fractions contained a higher protein/DNA ratio than those fractions in the upper part of the gradient. This was suggested from the parallel experiment using nuclei isolated from MSB-1 cells which were labeled with [^{14}C]thymidine and [^{3}H]lysine.

Electron microscopic observation of the DNA in the fast-sedimenting fractions revealed that a great majority of the DNA molecules contained no detectable protein association (not shown). Therefore, the fast-sedimenting fractions were passed through a glass fiber filter according to the procedure described by Coombs & Pearson (1978). These authors reported that the protein–DNA complex of adenovirus 2 DNA and its terminal protein (55 000 daltons) is retained by the GF/C filter, while the protein-free DNA passes through the filter. Using the same technique, Thomas et al. (1979) were able to isolate the protein–DNA complex from ϕ29 bacteriophage. There is little specificity of protein retention on the filter, since greater than 90% of total cellular proteins from *Bacillus substilis* can be recovered. The binding of protein-free DNA to the filter is generally very low (less than 0.03%). This was also confirmed with nuclear proteins from chicken erythrocytes and purified DNA from the same source. It was found that most (>95%) of the nuclear proteins were retained on the filter, and conversely, less than 0.02% of the purified DNA adhered to the filter (not shown). Encouraged by these observations, the filter binding procedure was used for the DNA–TBP isolation.

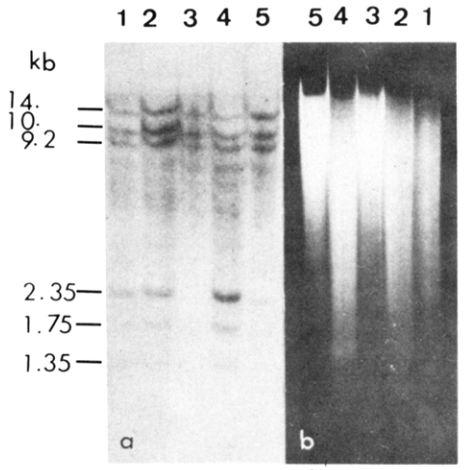

FIGURE 4: Detection of the *Eco*RI fragments in the ovalbumin gene region in the DNA samples prepared from chicken erythrocyte nuclei according to the procedure shown in Figure 1 scheme b: (a) autoradiogram; (b) ethidium bromide staining pattern of the gel before blotting. Lanes 1 and 2, total unfractionated DNA digested by *Eco*RI; lane 3, GF/C filter-bound DNA; lane 4, DNA in slow-sedimenting fraction; lane 5, filter-unbound fraction. Lanes 2–5 contain 5 μg of DNA; lane 1 contains 2.5 μg of DNA.

When chicken erythrocyte nuclei were used to isolate the DNA–TBP according to scheme a as shown in Figure 1, only about 0.2–0.4% of the DNA was retained on the filters (three experiments). The DNA that passed through the first filter was not retained when it was passed through a second filter. Virtually the same amount of DNA was retained on the filter when the nuclei isolated from either laying hen oviducts or livers were used. In conclusion, according to the present preparative scheme, the majority of the *Eco*RI fragments in the chicken genome are free of the tightly bound proteins.

Analysis of the Ovalbumin and Its Related Genes in the DNA Containing Tightly Bound Proteins. The ovalbumin gene and its neighboring DNA sequences approximately 50 kb in length have been isolated by the molecular cloning technique (Gannon et al., 1979; Royal et al., 1979). Two ovalbumin-related genes, designated X and Y, have been found to be located at the 5′ side of the ovalbumin gene. The probes used in this study were from two recombinant DNA clones, λC4-ov5 and X-4. The λC4-ov5 clone has a 16-kb insert that contains the entire ovalbumin gene and can be used to detect the *Eco*RI fragments 14, 9.2, 2.35, and 1.75 (or 1.35) kb. The X-4 clone contains a 2.3-kb insert located at the 3′ side of the X gene. This probe can be used to detect the *Eco*RI 10-kb or 3.5-kb fragments, depending upon the available *Eco*RI site, which is located within the *Eco*RI 10-kb fragment. [See Gannon et al. (1979) and Royal et al. (1979) for the locations of these restriction fragments in the ovalbumin gene family.]

The DNA fragments from the chicken erythrocyte nuclei prepared according to the fractionation scheme a shown in Figure 1 were separated by agarose gel electrophoresis, transferred to a nitrocellulose filter, and hybridized with ^{32}P-labeled DNA probes prepared from λC_4-ov5 and X-4 DNA clones. Figure 4 shows an autoradiogram and ethidium bromide staining pattern of a gel containing DNA from the GF/C filter-bound fraction (lane 3), the filter-unbound fraction (lane 5), and the slow-sedimenting fraction (lane 4). Total unfractionated chicken erythrocyte nuclear DNA digested by *Eco*RI was also included in the same gel (lanes 1 and 2) for comparison. Apparently, the contents of the *Eco*RI 14-, 10-, and 9.2-kb fragments in these DNA samples were

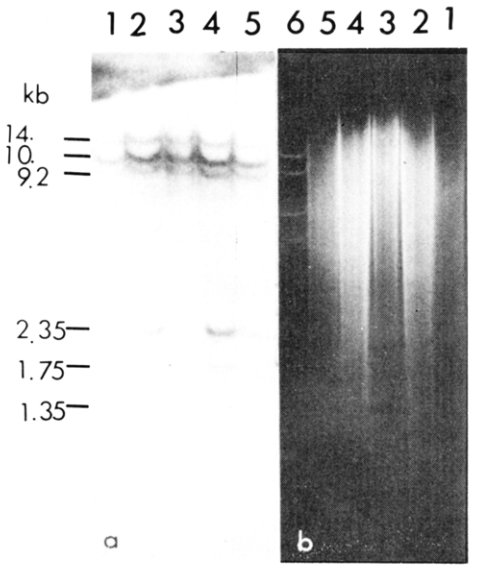

FIGURE 5: Detection of the *Eco*RI fragments in the ovalbumin gene region in the DNA samples prepared from chicken erythrocyte nuclei according to the procedure shown in Figure 1 scheme b: (a) autoradiogram; (b) ethidium bromide staining pattern of the gel. Lanes 1 and 2, total unfractionated DNA; lane 3, filter-bound DNA; lanes 4 and 5, filter-unbound DNA. Lanes 2–4 contain 5 μg of DNA, lanes 1 and 5 contain 2.5 μg of DNA. Lane 6, adenovirus 2 DNA digested by *Bam*HI.

not significantly different. This was determined by a comparison of the intensities of each band in the autoradiogram by densitometry (not shown). The amounts of *Eco*RI 2.35-, 1.75-, and 1.35-kb fragments in the filter-bound fraction were significantly lower than those in the slow-sedimenting fraction and those in the total unfractionated DNA. However, the ethidium bromide staining pattern of the gel also shows that the filter-bound fraction contained a diminished amount of small molecular weight DNA (less than 5000 base pairs). These results suggest that the distribution of DNA–tightly bound proteins in the chicken ovalbumin gene region is random.

The most reasonable explanation for the depletion of low molecular weight DNA in the filter-bound and filter-unbound fractions shown in Figure 2 is that the sucrose gradient sedimentation was used prior to the filter binding in the preparative scheme (Figure 1, scheme a). An alternative procedure for directly applying the *EcoRI*-digested DNA sample obtained from the Sepharose column to the filter binding was therefore used (Figure 1, scheme b). The result of this experiment showed that again only about 0.5% of the DNA was bound to the filter. Consistent with the result shown in Figure 4, the filter-bound DNA was not enriched (or depleted) in the sequences in the ovalbumin gene family (Figure 5a). Although the ethidium bromide staining pattern of the gel (Figure 5b) showed that the filter-bound fraction again contained a diminished amount of small molecular weight DNA, the general conclusion that the TBP are randomly distributed in the genome holds, since the high molecular weight DNA has a higher probability of containing the proteins.

Sequence Complexity Analysis of the DNA Containing the Tightly Bound Proteins. Solution hybridization using radioactively labeled DNA to hybridize with a large excess of unlabeled DNA allows one to determine if the labeled DNA represents a unique class of DNA in the genome. The filter-bound DNA, filter-unbound DNA (prepared as shown in Figure 1, scheme b), and total genomic DNA were labeled in vitro with ^{32}P by nick translation. Each labeled DNA gave a consistent single-stranded length of approximately 600 nucleotides. This was analyzed by agarose (1.6%) gel electrophoresis in alkaline conditions (30 mM NaOH and 2 mM EDTA) with ^{32}P-end-labeled *Hin*dIII-digested SV40 DNA as molecular weight markers (data not shown). Each labeled DNA was then annealed with a large (10^7-fold) excess of unlabeled, sonicated chicken DNA (approximately 500 nucleotides long). Shown in Figure 6 are the hybridization curves of this experiment. All three different hybridization reactions reached a similar plateau value of about 62% at a C_0t of 10000 [C_0t is the initial concentration of DNA (moles of nucleotide per liter) × time (seconds)]. At a C_0t of 10, approximately 20% of the labeled total genomic DNA and the filter-unbound DNA were in double-stranded forms, while about 25% of the labeled filter-bound DNA was hybridized to the total genomic DNA. All three hybridization reactions show approximately 40% of the labeled DNA annealing to the total nuclear DNA, resembling that for nonrepetitive sequences with $C_0t_{1/2}$ of 600. These results suggest that both filter-bound and filter-unbound DNAs, like the chicken genomic DNA, contain at least 60% of the nonrepetitive sequences, after a normalization of each hybridization reaction to 100% completion. The small amount of enrichment in middle repetitive sequences in the filter-bound DNA is probably not significant enough to indicate that the DNA containing TBP represents a unique class of genomic DNA.

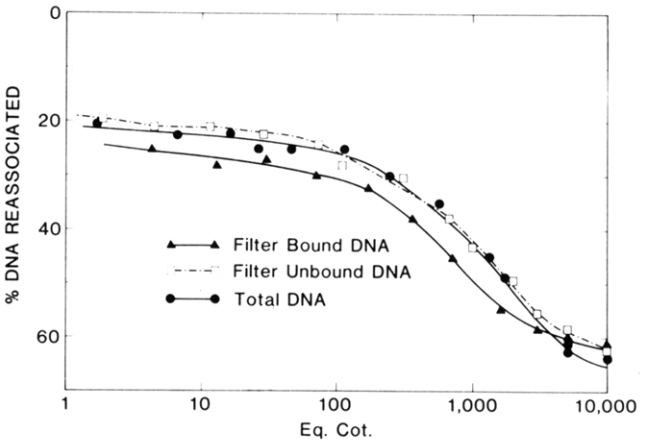

FIGURE 6: Time course of annealing of [^{32}P]DNA with sonicated nuclear DNA prepared from chicken erythrocyte nuclei according to the procedure described in Figure 1 scheme b. (▲) Filter-bound DNA; (□) filter-unbound DNA; (●) control, total unfractionated DNA.

The Ovalbumin Gene but Not the β-Globin Gene Is Significantly Enriched in the DNA Containing the Tightly Bound Proteins in Chicken Oviduct Nuclei. The results described above of a random distribution of the TBP in the genome of chicken erythrocytes are also consistent with those obtained when the nuclei from chicken livers, lymphoblastoid (MSB-1) cells, and metaphase chromosomes from the MSB-1 cells were used (data not shown). However, when the nuclei isolated from laying hen oviducts were used and subjected to the procedure shown in Figure 1 scheme b, an enrichment of the *Eco*RI fragments in the ovalbumin gene region in the filter-bound DNA was observed (Figure 7). Densitometric analysis of the autoradiogram, which was kept from being overexposed, revealed that the enrichment was approximately 3-fold. Conversely, the β-globin gene sequences were not enriched in the DNA–TBP prepared from the oviduct nuclei (Figure 8). This is evident from the fact that neither the embryonic (at the junction between the 9.4- and 6.0-kb fragments) nor the adult (in the 6.0-kb fragment) β-globin gene in the filter-bound fraction was significantly different from that in the total un-

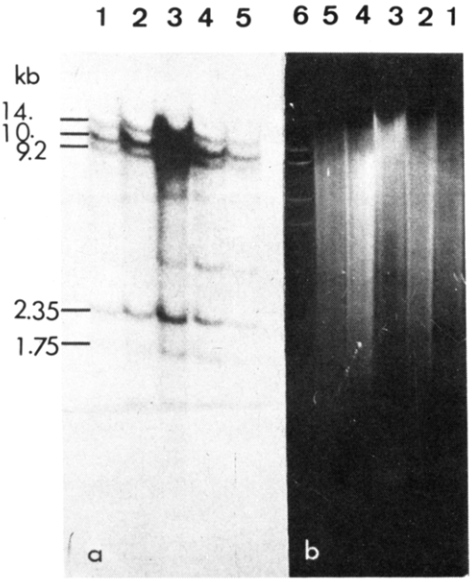

FIGURE 7: Analysis of the *Eco*RI fragments in the ovalbumin gene region in the DNA samples prepared from laying hen oviduct nuclei following the procedure shown in Figure 1 scheme b: (a) autoradiogram; (b) ethidium bromide staining pattern of the gel. Lanes 1 and 2, total unfractionated DNA; lane 3, filter-bound DNA; lanes 4 and 5, filter-unbound fraction; lane 6, adenovirus 2 DNA digested by *Bam*HI. Lanes 2–4 contain 5 μg of DNA; lanes 1 and 5 contain 2.5 μg of DNA.

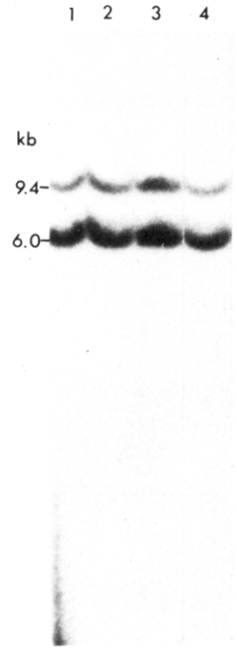

FIGURE 8: Analysis of the *Eco*RI fragments in the β-globin gene region in the DNA samples prepared from laying hen oviduct nuclei according to the procedure shown in Figure 1 scheme b. Lanes 1 and 4, total unfractionated DNA; lane 2, filter-bound DNA; lane 3, filter-unbound DNA. Each lane contains 5 μg of DNA. The probe used in this experiment was from the pBR322-βG1 clone (Bellard et al., 1980).

fractionated chicken nuclear DNA [see Dodgson et al. (1979) for the restriction map of the β-globin genes]. Since the ovalbumin gene but not the globin gene is expressed in laying hen oviduct, this result is consistent with reports by other investigators (Gates & Bekhor, 1979a,b; Bekhor & Mirell, 1979; Ross et al., 1979) that the transcribing genes are enriched in the DNA which contain tightly bound proteins. I have reservations about this conclusion, however (see Discussion), since the DNA–TBP in the nuclei of laying hen oviducts is about 0.5% of the total DNA; a great majority (~99%) of the gene sequence is still in the DNA that is devoid of the TBP.

Discussion

In this paper, a method for isolating the DNA associated with the TBP is described. This method seems to be very effective, because of the filter binding step. The filter binds DNA containing proteins but not protein-free DNA. Most of the published procedures for preparations of DNA–TBP involved repeating centrifugations and designation of the "fast-sedimenting fractions" as the ones containing TBP. However, these manipulations do not ensure that the fast-sedimenting fractions contain only protein–DNA complexes. The high molecular weight DNA may cosediment with the DNA–TBP complex (unpublished observations). Moreover, the present procedure includes a chromatographic step that should efficiently eliminate the contamination of loosely bound proteins from DNA.

The major point presented in this report is that, using the present procedure, only a small fraction (0.5%) of the *Eco*RI fragments in the chicken genome contain the TBP. This value is consistent in all cells irrespective of the tissue origin or stage of the cell cycle (i.e., interphase vs. metaphase). This fraction of DNA is not a specific sequence class in the genome as determined by liquid hybridization. Southern blots also revealed that, except in the laying hen oviduct, the ovalbumin gene sequence is not enriched in the DNA–TBP fractions.

In the laying hen oviduct in which ovalbumin genes are actively transcribed, a 3-fold enrichment of the ovalbumin gene sequences in the DNA containing the tightly bound proteins was detected. Several reports have been published showing that the transcribing gene sequences are enriched in the DNA that contains tightly bound proteins (Gates & Bekhor, 1979a,b; Bekhor & Mirell, 1979; Ross et al., 1979; Norman & Bekhor, 1981). These authors proposed that the TBP are important regulatory molecules for gene expression. However, it is reasonable to assume that the DNA–TBP isolated from the chromatin may contain factors for transcription or subunits of RNA polymerases, since the transcribing complexes are resistant to high molar salt (Gariglio et al., 1979). It has been reported that heterogeneous nuclear RNA (hnRNA) remained tenaciously associated with the nuclear matrix proteins (Long et al., 1979), which may be in part related to the TBP discussed here. Recently, Jackson et al. (1981) have reported that DNA associated with the nuclear matrix from HeLa cells is highly enriched in transcribing sequences, as demonstrated by hybridizations of the total DNA and the matrix DNA to an excess of nuclear RNA. Using pulse-labeling experiments, these authors also demonstrated that nascant RNA is synthesized at the nuclear matrix. It has also been found that the DNA–TBP collected on the filters contains a significant amount of RNA (unpublished observation). In this respect, it is not surprising to see that the TBP contain an elevated amount of transcribing gene sequences. Therefore, care needs to be taken in drawing the conclusion that the TBP are regulatory elements of gene expression. Taking this into account, the DNAs associated with the TBP in both transcribing chromatin as well as nontranscribing chromatin are not sequence specific.

It has been suggested that the TBP may be related to the proteins in the nuclear matrix (Berezney & Coffey, 1975) or in the chromosome scaffold (Laemmli et al., 1977), because both the matrix and the scaffold have been prepared by passing the nuclei or chromosomes through high molar salt. The TBP discussed in this report may not represent the entire matrix

or scaffold proteins, because a rather harsh procedure (i.e., shearing of chromatin) was used. (In fact, neither nuclear matrix nor chromosomal scaffold proteins have been well characterized.) A mild procedure which involved repeating centrifugations of unsheared nuclei or isolated chromosomes through sucrose gradients containing 2 M NaCl was also used, and the same result, i.e., a random distribution of the TBP in the ovalbumin gene region (and the total genome DNA), was obtained (Kuo, 1981). Recently, Nelkin et al. (1980) have reported a 3–7-fold enrichment of SV40 DNA relative to the total cellular DNA in the DNA intimately associated with the nuclear matrix prepared from the SV40-infected mouse 3T3 cells. These authors also reported that the mouse α- and β-globin genes are evenly distributed in the nuclear matrix and nonmatrix DNA. My interpretation of their results is the following: like that shown in Figure 7, the transcribing gene (SV40 DNA in the transformed cells) is enriched in the DNA associated with the nuclear matrix, possibly due to copurification of the transcriptional complexes, and the nontranscribing genes (the α- and β-globin genes), like that shown in Figure 8, are randomly distributed throughout the genome. It is presently premature to conclude that the SV40 DNA resides near the matrix–DNA attachment point, as these authors claimed.

Two possibilities can be suggested for interpreting the mechanisms for the random distribution of TBP in the genome. (1) The interaction of the TBP with DNA may be a transient phenomenon. Recent results from other laboratories (Pardoll et al., 1980; Berezney & Buchholtz, 1981; McCready et al., 1980; Vogelstein et al., 1980) suggested a dynamic interaction of DNA and protein in the nuclear matrix—at least during the DNA replication process. In this regard, the association of DNA with TBP may not be as tight as one might have thought. Direct proof of this point requires further investigations. (2) The procedures described in this report, as well as in most of the literature dealing with DNA–TBP or nuclear matrix, do not preclude the possibility of a redistribution of TBP in DNA. If a randomization of the TBP in the genome is taking place during the manipulations, one would not be surprised to see that there is no specific DNA sequence associated with the tightly bound proteins. In this regard, those papers that deal with the nuclear matrix, nuclear skeletons, etc. need to be interpreted with great care. Monoclonal antibodies against the TBP are currently being prepared, and I wish through immunocytochemical studies to investigate these possibilities.

Acknowledgments

I thank Professor P. Chambon for his hospitality and many helpful suggestions. I also thank Dr. A. Kindelis for his help in electron microscopy in some of the DNA samples and Ruby Kirkpatrick for her clerical help in the preparation of this manuscript.

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