with dimer progressively weakens; the contribution from other types of bonding therefore declines very sharply indeed. This points to the free-energy contribution from other types of noncovalent interactions being responsible for the enhanced stability of the region, particularly at short filament lengths.

The discovery of a progressive decrease in the $\Delta G°$ of interaction within a structure of comparable dimensions to that of the minifilament makes it unlikely that they function as a nucleus of the thick filament in the accepted sense of the word. As was pointed out, the reinterpretation of the experiments on de novo thick filament formation points to the nucleation reaction occurring between a pair of parallel dimers, an event which occurs very early in the assembly process. It remains to be discovered how this intermediate with its very high affinity for dimer is built.

It has recently been shown that different types of myosin molecules can be localized in different parts of the thick filament (Miller et al., 1983). This implies a selection mechanism of some sort. As we have seen, quite different bonding interactions occur in the parallel- and antiparallel-packed regions of the thick filament. A particular myosin variant might thus have a high affinity for assembly into the bare zone through a particular set of interactions and low affinity for assembly into the parallel-packed arms.

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Chromatin Structure of a 3-Methylcholanthrene-Induced Cytochrome P-450 Gene†

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ABSTRACT: Plasmids carrying fragments of a cytochrome P-450 gene, inducible by 3-methylcholanthrene, were used to study the chromatin structure of this gene in the liver of normal and carcinogen-treated rats. Digestion with micrococcal nuclease revealed that the gene is not present in the typical 200 base pair nucleosomal structure. By use of indirect end-label hybridization, four DNase I hypersensitive sites were mapped in the 5′-terminal region of the gene. An S1 nuclease sensitive site is located close to a DNase I site. Gene induction by treatment with 3-methylcholanthrene does not result in detectable changes in the DNase I hypersensitive sites. Rat thymus chromatin does not contain DNase I hypersensitive sites in the P-450 gene, suggesting that in the liver the chromatin structure is altered so as to allow tissue-specific expression of the gene. This paper is the first study on the chromatin structure of a gene coding for a member of the cytochrome P-450 family of enzymes. The implications of our results to the understanding of gene regulation of the P-450 genes are discussed.

The cytochrome P-450 family of proteins is responsible for metabolizing a variety of xenobiotic and endogenous compounds including drugs, carcinogens, and toxins, (Conney, 1982). Some of the metabolites produced are potent mutagens and carcinogens which bind covalently to cellular macromolecules such as proteins, RNA, and DNA (Gelboin, 1980). Syntheses of the various members of the cytochrome P-450 enzyme system are highly inducible in a very selective manner, and the isoenzymes generated have distinct yet overlapping substrate specificities (Lu & West, 1980). Gene expression regulation, therefore, is central to the coordinate control of this family of genes.

Compared to total nuclear DNA, active or potentially active genes have an altered chromatin structure which can be examined with various nucleases [for a review of chromatin structure and nuclease sensitivity, see Igo-Kemenes et al. (1982) and Elgin (1981)]. Transcribable regions are preferentially digested with DNase I presumably because of an

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altered chromatin structure. The relationship between gene expression and the DNase I sensitivity is observed not only in expressed genes but also in those genes which have been transcribed and those which retain the potential for transcription. Another feature of chromatin structure revealed by DNase I is the so-called hypersensitive site. These regions near active genes are hypersensitive to digestion by the nuclease DNase I and change their pattern of sensitivity corresponding to changes in gene expression. For example, hypersensitive sites in the erythroblast β-globin genes appear to an identifiable stage during development and prior to β-globin synthesis (Weintraub et al., 1981; McGhee et al., 1981). The appearance of hypersensitive sites can be correlated to the induction of transcription of heat-shock genes in *Drosophila* (Wu, 1980) and immunoglobin gene rearrangement in early pre-B cell lymphocytes (Rose & Garrad, 1984). The DNase I hypersensitivity patterns of the translocated myc gene allele in Burkitt lymphoma are correlated with myc gene expression (Siebenlist et al., 1984). DNase I hypersensitivity can be conferred by protein binding near the hypersensitive site as demonstrated in a reconstituted system (Emerson & Felsenfeld, 1984). Preferential binding of specific cellular proteins to sequences containing hypersenitive sites can be demonstrated in vitro in the *Drosophila* heat-shock gene *hsp*82 (Wu, 1984).

Two other nucleases, single-strand-specific nuclease S1 and micrococcal nuclease, have also been used to examine the chromatin structure of specific genes. S1 preferentially digests sites which have been correlated with gene expression both in chromatin (Larsen & Weintraub, 1982) and in plasmids containing genomic sequences (Hentschel, 1982; Schon et al., 1983; Cockerill & Goodwin, 1983). These regions appear to have an alternate DNA conformation which results in preferential S1 digestion and which may provide a basis for DNA-specific protein binding. Digestion of nuclei with micrococcal nuclease has been used to examine whether a specific gene is in the nucleosomal conformation typical of most of the genomic DNA [for a review, see Igo-Kemenes et al. (1982)].

In the present paper, we examine the chromatin structure of a cytochrome P-450 gene which is constituitively expressed at relatively low levels in the uninduced rat liver and which is induced approximately 4-fold by intraperitoneal injection of 3-methylcholanthrene (Fagan et al., 1982). DNase I, micrococcal nuclease, and S1 nuclease were used to examine the chromatin conformation of this gene in induced and noninduced rat liver nuclei.

Materials and Methods

Preparation of Nuclei. Male Sprague-Dawley rats weighing 160–180 g were obtained from the NIH small-animal facility in Frederick, MD. Induction of cytochrome P-450M was achieved by injection of 20 mg of 3-methylcholanthrene (Tridon Chemical Co., Hauppauge, NY) in corn oil per kilogram rat body weight into the peritoneal cavity. Following a 24-h induction period, the rats were sacrificed, and the liver was excised and cooled on ice. All subsequent steps except enzyme digestions were done at 4 °C. Nuclei were isolated by using modifications of the technique of Chauveau et al. (1956) and Blobel & Potter (1966). Minced liver tissue was homogenized in 0.25 M sucrose, 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.5), 25 mM KCl, and 5 mM $MgCl_2$ with a Teflon–glass pestle, mixed with sucrose to 1.6 M, and pelleted through 2.3 M sucrose in the same buffer at 124000*g* in a Beckman SW28 rotor for 45 min. The nuclei were resuspended in the appropriate digestion buffer at 0.5 mg/mL.

Nuclease Digestions. DNase I (Worthington) digestions were done in 0.34 M sucrose, 50 mM Tris-HCl (pH 7.5), 10 mM NaCl, and 3 mM $MgCl_2$ using varying amounts of enzyme for 10 min at 37 °C. Micrococcal nuclease (Worthington) digestions were done in 10 mM Tris-HCl (pH 8.0) and 1.0 mM $CaCl_2$ at 37 °C for 10 min; the digestion was stopped by adding sodium ethylenediaminetetraacetic acid (Na EDTA) to 5 mM. The nuclei were pelleted away from nucleosomes released into the supernatant by centrifugation at 10000 rpm in a Sorvall HB-4 rotor. The DNA was isolated by proteinase K digestion (BRL) overnight at 37 °C, digested with RNase A (Sigma), and subsequently purified by phenol and chloroform extractions. The chromatin DNA was then extensively dialzyed in 10 mM Tris-HCl and 2 mM EDTA, pH 7.5. Single-strand-specific nuclease S1 (BRL) was used to digest supercoiled plasmids in 30 mM sodium acetate and 1 mM zinc acetate, pH 4.5, with 0–200 mM NaCl. Restriction enzymes were obtained from BRL and New England BioLabs and used as recommended by the manufacturer.

Electrophoresis, Transfer, and Hybridization. DNA samples were separated on 3-mm vertical 1% or 1.5% agarose gels by using a Hoeffer SE600 with one frosted plate per gel. Gels were run (45 V) overnight at 4 °C in Tris–acetate buffer, pH 8.4.

Following ethidium bromide staining, the gels were photographed and the DNA was acid-nicked by incubating the gel in 0.25 M HCl for 15 min and denatured by incubation in 0.5 N NaOH and 1.5 M NaCl for 40 min. Transfer to Gene Screen Plus (New England Nuclear) was done in 12 mM Tris-HCl (pH 7.5), 6 mM sodium acetate, and 0.3 mM EDTA using a Hoeffer Transfer at 10 V for 45 min followed by 60 min at 40 V at 4 °C. The nylon membrane was air-dried, prehybridized in 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate (Pharmacia), 50 mM Tris-HCl (pH 7.5), and 1 M NaCl overnight at 65 °C, and probed with 10^7 counts of ^{32}P-labeled plasmid probe [nick translated to $(1–2) \times 10^8$ counts/μg of DNA by using the BRL nick-translation kit and Amersham dCTP] with 100 μg/mL denatured salmon sperm DNA (Sigma) as carrier. Plasmid fragments used for hybridization were isolated by horizontal transfer to DEAE-cellulose (Schleicher & Schuell) in a Hoeffer Transfer device using the procedure of Danner (1982). Nick-translated *Hind*III cut λ-phage (BRL) was cohybridized as a radioactive marker. Hybridization proceeded for 20–48 h at 65 °C with agitation. The membrane was washed with 2× SSC (SSC = 150 mM NaCl and 15 mM sodium citrate, pH 7.0) at room temperature for 10 min, 2× SSC with 1% SDS at 65 °C for 60 min, and finally with 0.1× SSC at room temperature for 60 min. The washed membrane was blotted dry and autoradiographed by using Lighting Plus intensifying screens (Du Pont). Rehybridization was performed by allowing the probe to decay and then repeating the prehybridization and hybridization steps. Slot blots were done by using Gene Screen Plus in a slot blot apparatus (Schleicher & Schuell) as recommended by the manufacturer.

Plasmids. The 3-methylcholanthrene-induced P-450M gene was isolated from a rat genomic library prepared by T. Sargent. Subclones suitable for indirect end-label hybridization were prepared as described by J. Fagan and M. Bustin (unpublished results). Plasmids were cloned in pBR322 with *Echerichia coli* strain HB101. Plasmid amplification was done in rich medium containing 240 μg/mL chloramphenicol. Bacteria were lysed by using the Triton X-100 method of Katz et al. (1973), and plasmids were isolated by using the double banding method of Radloff et al. (1967). The NIH guidelines for recombinant DNA research were followed throughout the

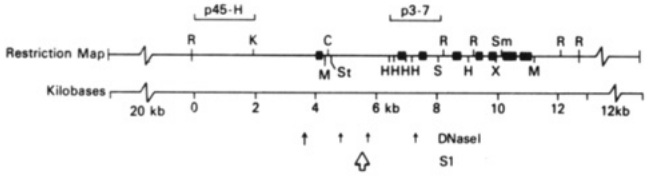

FIGURE 1: Genomic map of the P-450M gene. A 12.4-kb genomic fragment contains the entire coding region of the P-450M gene and approximately 4 kb upstream and 2 kb downstream. Regions that are transcribed into mRNA are indicated by boxes. Restriction enzyme digestion sites are indicated by the following letters: R, *Eco*RI; K, *Kpn*I; M, *Mst*II; C, *Cla*I; St, *Stu*I; H, *Hin*dIII; S, *Sst*I; X, *Xho*I; Sm, *Sma*I. Two genomic subclones which were used for hybridization, p45-H and p3-7, are indicated. Arrows indicate the DNase I hypersensitive sites and the S1 digestion site as determined by the work presented in this paper.

work. Plasmid pRSA57, the rat albumin gene, was obtained from T. Sargent (Sargent et al., 1979), plasmid pI-19, the rat insulin gene, from P. Lomedico (Villa-Komaroff et al., 1978), andd plasmid p4D-12, a rat repetitive sequence, from A. T. Furano (Whitney & Furano, 1984).

RESULTS

We have used nucleases to examine the chromatin structure of the P-450M gene prior to and following induction of transcription by treatment of rats with 3-methylcholanthrene. A genomic map of the gene is diagrammed in Figure 1. The entire P-450M gene is contained within a 12.4-kilobase (kb) genomic fragment. Coding regions are indicated by the black boxes. Restriction enzyme *Eco*RI cuts the P-450M insert at five sites. Plasmid p45-H contains 2000 base pairs and is located approximately 2000 base pairs upstream of the start of P-450M transcription. Plasmid p3-7, which spans the central portion of the gene, contains 1800 base pairs, including two exons. Both subclones abut an *Eco*RI restriction site.

In the first set of experiments, we looked for changes in the overall DNase I digestibility of P-450M DNA following induction and compared the observed change in digestibility to those occurring in other rat genes. In the slot blot (Figure 2), the same amount of DNA has been applied to each well. As digestion proceeds, the DNA fragments become sufficiently short so that they will not bind to the nylon membrane. The rate of digestion of bulk DNA can be followed by probing with plasmid p4D-12, which contains repetitive DNA sequences.

The rate of digestion of an untranscribed gene was followed by probing the filter with plasmid pI-19, which contains a portion of the rat insulin gene. The DNase I sensitivity of this gene is very similar to that of bulk DNA. Similar results were obtained with pRSA57, a plasmid containing a portion of the rat albumin gene. The blots were also probed with two plasmids containing different regions of the P-450M gene (see Figure 1). Plasmid p45-H contains a noncoding 2000 base pair region upstream of the 5′ end of the gene. The DNA hybridizable to these plasmids is more DNase I sensitive than bulk DNA or the insulin gene; however, the DNase I sensitivity is not affected by induction of the gene. In contrast, the exon which hybridizes to plasmid p3-7 is somewhat more digestible with DNase I in the induced nuclei as compared to uninduced nuclei (compare radioactive signal of DNA digested with 2 μg/mL DNase I in uninduced liver to that of 1 μg/L in induced liver).

Thus, our data indicate that the DNase I sensitivity of genomic DNA and the albumin and insulin genes is unaffected by treatment of rats with 3-methylcholanthrene while the coding region of the cytochrome P-450M gene is slightly more sensitive. The DNase I sensitivity of an untranscribed region

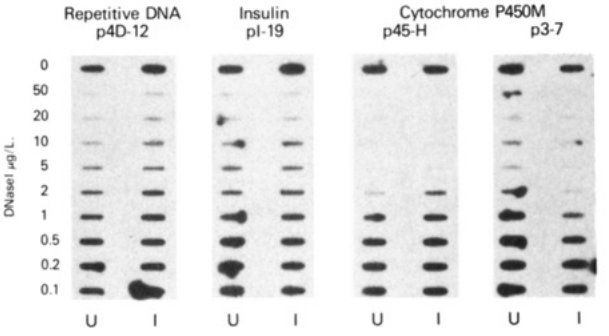

FIGURE 2: Altered DNase I sensitivity in a P-450M exon detected by slot blot analysis. Equal amounts of DNA from either induced (I) or uninduced (U) rats were digested with the concentration of DNase I indicated on the left, applied to a nylon membrane, and subsequently probed with either plasmid p4D-12, a genomic rat repetitive gene, plasmid pI-19, which contains a portion of the rat insulin gene, plasmid p45-H, which is located 2000 bp upstream of the start of transcription, or plasmid p3-7, which contains coding regions of the P-450M gene. Only plasmid p3-7 demonstrates increased sensitivity to DNase I following induction.

which is less than 2000 base pairs upstream of the 5′ end of the cytochrome P-450M gene is unaffected by induction.

Since changes in DNase I hypersensitive sites have been shown to be associated with changes in gene expression, we next analyzed the location of such sites in the chromatin present in rat liver nuclei isolated from both normal and 3-methylcholanthrene-induced rats. In these experiments, isolated nuclei are briefly digested with DNase I, and the digested DNA is isolated, restricted with *Eco*RI, and fractionated on an agarose gel. The DNA fragments are transferred to a nylon membrane which is probed with ^{32}P-labeled fragment which allows mapping of preferred DNase I digestion sites relative to the known restriction map of the gene (see Figure 1).

As can be seen from the ethidium bromide stained gel in Figure 3A, increasing DNase I digestion dramatically reduces the size of the DNA (0–20% acid-soluble nucleotides). In the first four lanes of the autoradiogram, however, the intact *Eco*RI/*Eco*RI gene fragment of 8.2 kb can be identified (see arrow). The probe used is p3-7, and 1800 base pair fragment which abuts the *Eco*RI restriction enzyme site at 8.2 kb, and therefore can be used to map preferential DNase I sites by measuring the length of the newly generated DNA fragment upstream from that restriction site. Using this technique, we have identified four DNase hypersensitive sites in the 5′ region of the gene (arrowheads, Figure 3B; diagram, Figure 1). The most prominent 5′ DNase I hypersensitive site is 500 base pairs 5′ to the first transcribed region. Three less prominent DNase I hypersensitive sites are located in the first two introns of the P-450M gene. Following induction, the same four hypersensitive sites can be seen (arrowheads, Figure 3C; arrows, Figure 1).

Hypersensitive sites can also be measured from the 3′ end of the genomic clone in an analogous fashion using plasmid p45-H as a probe (Figure 4). An ethidium bromide stained gel demonstrates the DNase I digestion (Figure 4A). Hybridization with plasmid p45-H, which abuts the *Eco*RI site at 0 base pairs (bp) (Figure 1), allows mapping of hypersensitive sites in both uninduced (Figure 4B) and induced (Figure 4C) nuclei. Because the DNase I site (3.6 kb in Figure 1) is the preferred digestion site, and because the other DNase I fragments are poorly resolved on the gel, the sites downstream from the 3.6 kb site are difficult to identify (Figure 4B,C). The 10.2-kb fragment above the 8.2-kb *Eco*RI/*Eco*RI fragment in the lane corresponding to 0.1 μg/L results from incomplete *Eco*RI digestion. Minor DNase I hypersensitive sites

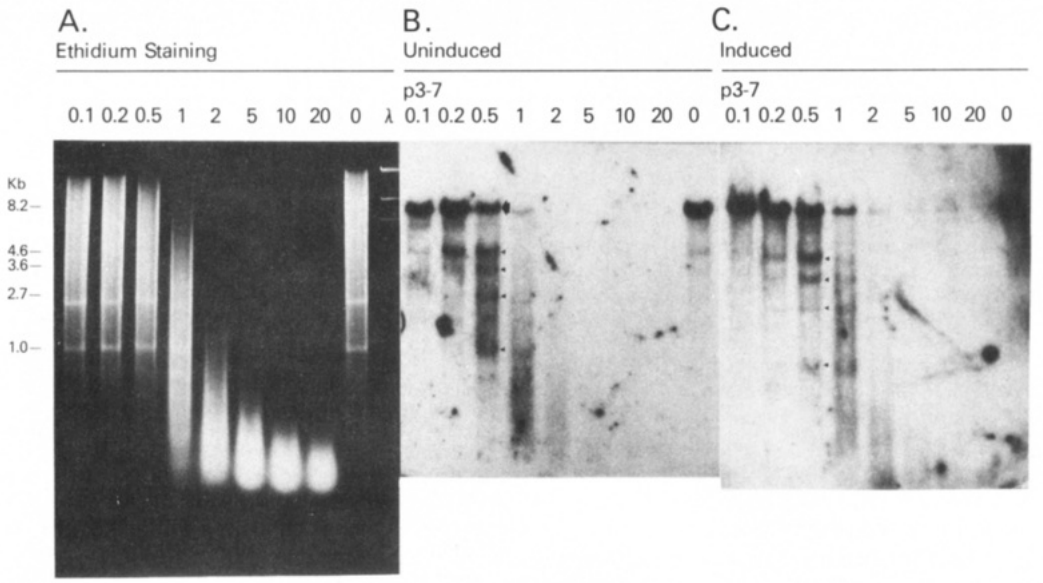

FIGURE 3: DNase I hypersensitive sites in the rat liver P-450M gene mapped with plasmid p3-7. (A) Ethidium stained gel prior to transfer demonstrates the digestion of the DNA with increasing concentrations of DNase I (numbers on top of columns indicate concentration of DNase I in micrograms per liter). Autoradiograms of DNA from uninduced (B) and induced (C) rats show the 8.2-kb, *Eco*RI/*Eco*RI band (large arrow) and the four bands generated by *Eco*RI/DNase I cuts at 4.6, 3.6, 2.7, and 1.0 kb (arrowheads). Some variation in the relative intensities of the bands following induction can be observed by comparing (B) with (C).

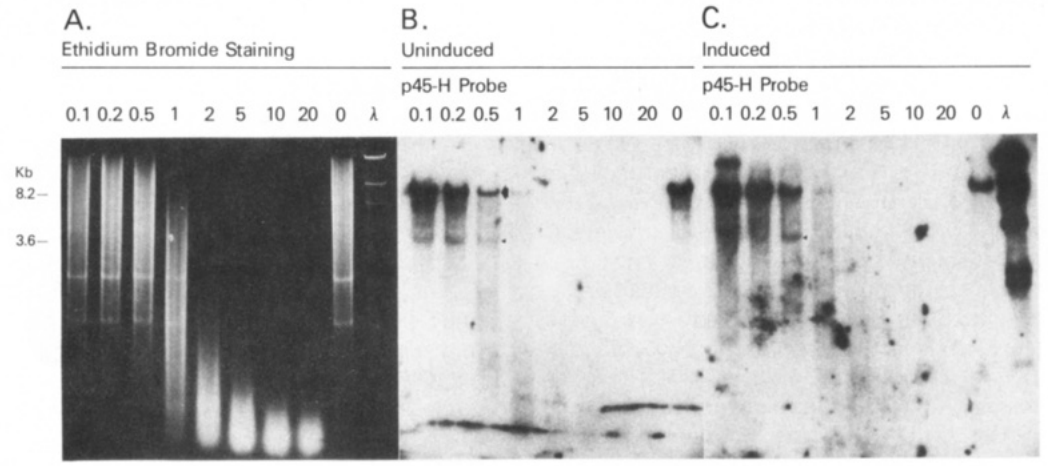

FIGURE 4: DNase I hypersensitive sites in the rat liver P-450M gene mapped with plasmid p45-H. The experiment was analogous to that of Figure 3 except that the plasmid hybridizes to the 5′ end of the *Eco*RI/*Eco*RI fragment (large arrow at 8.2 kb). A 3.6-kb *Eco*RI/DNase I band is evident (arrowhead). The minor DNase I sites detected in Figure 3 are less prominent and poorly resolved.

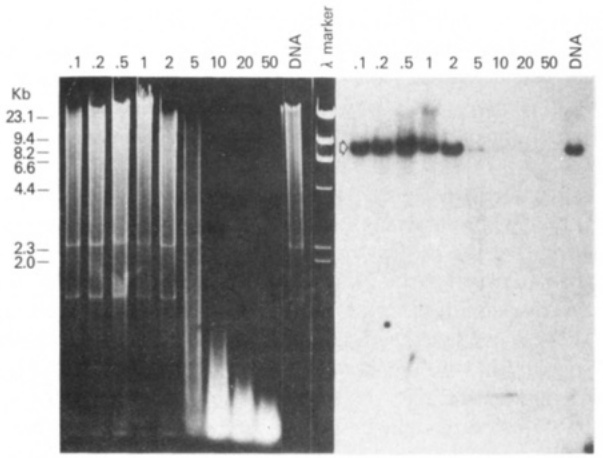

FIGURE 5: Lack of DNase I hypersensitive sites in the rat thymus P-450M gene. This experiment is analogous to Figure 3 except that the nuclei are obtained from the thymus. The large arrow marks the 8.2-kb *Eco*RI/*Eco*RI fragment. Note the absence of smaller fragments in the autoradiogram.

which were not affected by gene induction were detected upstream of the cloned gene by digesting chromatin with *Mst*II and probing with plasmid p45-H (data not shown).

In an analogous indirect end-labeling experiment, DNase I digestion of rat thymus nuclei demonstrates that the thymus chromatin lacks DNase I hypersensitive sites in the 5′ region of the P-450M gene (Figure 5). The lack of hypersensitive sites near the P-450M gene in the thymus indicates that in the liver the chromatin structure is altered in some manner so as to allow tissue-specific expression of this gene. DNase I displays a degree of sequence-specific digestion (Drew, 1984). However, this experiment and analogous experiments with purified rat DNA (data not shown) indicate that the DNase I digestion pattern observed in the liver nuclei reflects the specific nucleoprotein structure of this gene rather than its DNA sequence.

While 3-methylcholanthrene treatment did not cause the formation of additional DNase I hypersensitive sites in the P-450M present in the rat liver genome, the slot blot experiments demonstrated a slight increase in DNase I digestibility.

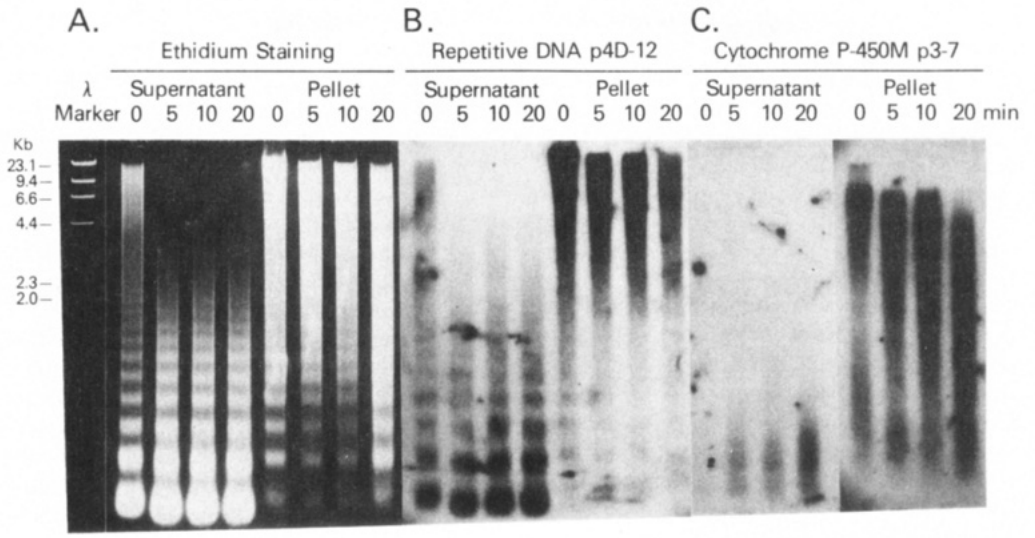

FIGURE 6: Micrococcal nuclease digestion of induced rat liver nuclei. The ethidium bromide stained gel (A) prior to transfer demonstrates the 200 bp nucleosomal ladder and the release of nucleosomes from the nucleus by the nuclease. Hybridization with plasmid p4D-12, a rat repetitive DNA (B), demonstrates the same pattern as the ethidium bromide stain (C). Hybridization with plasmid p3-7 reveals that P-450M gene in rat liver is in a nonnucleosomal conformation.

We sought, therefore, to examine the nucleosome structure of the gene in induced liver by digesting intact nuclei with micrococcal nuclease. Following nuclease digestion, the nuclease activity was quenched with EDTA, and the nuclei were sedimented. The nucleosomal patterns of the gene in the DNA released into the supernatant and remaining in the pellet were examined on 1.5% agarose gels.

The ethidium bromide staining of the DNA contained in the two fractions at different levels of digestion is shown in Figure 6. Both the supernatant and the pellet DNA displayed a typical nucleosomal pattern. The DNA from such gels was transferred to Gene Screen Plus and probed with either p4D-12 which contains repetitive rat sequences or plasmid p3-7 which contains a p-450M coding region. While the autoradiograph generated after probing with p4D-12 displayed a typical nucleosomal pattern similar to that produced by the ethidium bromide stain, the pattern generated by p3-7 was distinctly different. The supernatant fraction was significantly depleted in these coding sequences, and the radioactivity detected is a smear, suggesting that this region of the gene is not organized in a regular nucleosomal configuration. The pellet fraction is significantly enriched in P-450M sequences. Localization of these sequences in this insoluble chromatin fraction perhaps reflects an association with the nuclear matrix. The sequences associated with these fractions are also in a nonnucleosomal conformation. Probing with plasmid p45-H, which is located 2000 base pairs upstream of the transcribed gene, shows a similar pattern (data not shown), suggesting that the nonnucleosomal configuration extends beyond the 5′ terminus of the gene.

Next we determined whether this gene contains S1-sensitive sequences. Using the indirect end-labeling method, we have mapped S1 sites in a plasmid containing the entire 12.4-kb cytochrome P-450M gene. The fragments generated by S1 and *Eco*RI digestion were separated on a gel, transferred to a nylon membrane, and probed with the 1200 base pair *Eco*RI/*Hind*III fragment, isolated from plasmid p3-7, abutting the 8.2-kb *Eco*RI site (Figure 1). The assess the stability of the S1 site, the digestion was performed at NaCl concentration ranges from 0 to 300 mM. The results are presented in Figure 7. Digestion of plasmid 6A, which includes the entire P-450M gene shown in Figure 1, with *Eco*RI generated five fragments (Figure 7A). The labeled fragment hybridizes with the 3′ end

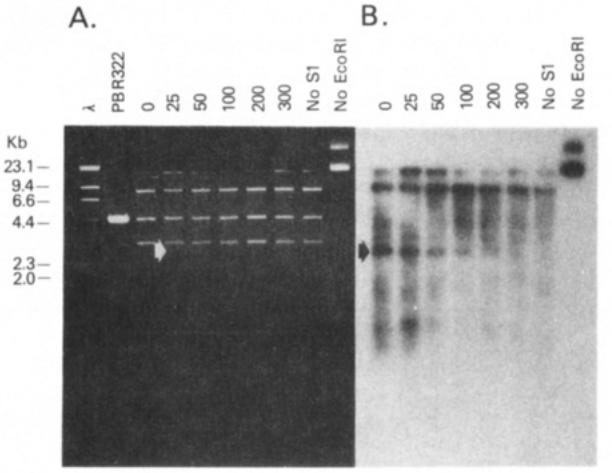

FIGURE 7: Mapping of the single-strand-specific enzyme S1 digestion site in the P-450M gene. S1 digestion of a plasmid containing the entire P-450M gene at various salt concentrations (0–300 mM NaCl) followed by *Eco*RI digestion results in a series of bands (see ethidium bromide stained gel A). Digestion with S1 results in a new band (see white arrow) which is clearly seen in the autoradiogram (B) following hybridization with the insert from p3-7 (see black arrow).

of the 8.2-kb fragment bracketed by *Eco*RI sites at 0 and 8.2 kb. After S1 digestion at low ionic strength, a band can be visualized in the ethidium bromide stained gel (see white arrow, Figure 7A) which is prominent in the autoradiogram (see black arrow, Figure 7B). This 2800 base pair S1/*Eco*RI-generated fragment maps very closely to a DNAse I hypersensitive site in intact chromatin (Figure 1). As can be seen in Figure 7B, the S1-specific sites are very sensitive to salt concentration during digestion. S1 digestion conditions have been chosen such that all supercoiled plasmids have been converted into linear molecules except in 300 mM salt where S1 activity is very low. We asked if the salt might be acting upon the plasmid by tending to melt or unwind the DNA (Hentschel, 1982). Modification of the plasmid superhelicity by incubating the plasmid in varying ethidium bromide concentrations in the presence of topoisomerase I and subsequent digestion of the plasmid by S1 revealed that changing the linking number had no effect upon S1 digestion near the site of the DNase I hypersensitive site (data not shown). We interpret these results to indicate that the decrease in S1 site

specificity observed with increasing salt concentration is not due to changes in the superhelicity of the plasmid.

The results of the enzymatic mapping digestions are diagrammed in Figure 1. The major DNase I hypersensitive site and the plasmid S1 site are both approximately 1 kb upstream of the first transcribed exon. Three other minor DNase I hypersensitive regions are located in the first and second introns.

Discussion

Regulation of expression in the physiologically important cytochrome P-450 family of genes is characterized by a highly specific induction of gene expression. The synthesis of P-450M mRNA is maximal 24 h after treatment of rats with 3-methylcholanthrene (Fagan et al., 1982). We have asked whether this induction is associated with an observable change in the chromatin structure of the P-450 gene. This paper is the first study of the chromatin structure of a member of the P-450 family of enzymes.

Micrococcal nuclease digestion of nuclei isolated from induced rats reveals that the chromatin structure of the P-450M gene is different from that displayed by the genome as a whole. The ethidium bromide stain of the DNA isolated from the supernatant and pellet of the digested nuclei reveals the expected 200 base pair nucleosomal ladder. The digestion was random since probing the digest with highly repetitive DNA (plasmid p4D-12) resulted in an autoradiograph which faithfully reproduced the ethidium bromide pattern. In contrast, the transcribed P-450M gene, probed with plasmid p3-7, is not organized in the same conformation. Futhermore, the DNA present in the pellet fraction is enriched in P-450M sequences. Nonnucleosomal DNA structure has been observed for other transcribed genes (Wu et al., 1979; Levy & Noll, 1981; Weischet et al., 1983; Bellard et al., 1982; Bloom & Anderson, 1982). Rose & Garrard (1984) suggested that the irregularity in nucleosome spacing reflects a deficiency in histone H1 and the presence of the gene in the pellet reflects its association with the nuclear matrix. However, these studies only suggest and do not prove this association.

Four DNase I nuclease hypersensitive sites have been detected in the rat liver chromatin in the 5′ region of the cytochrome P-450M gene. The pattern of nuclease sensitivity in these regions does not appear to change following induction of the gene with 3-methylcholanthrene. During this same period, there is a 4-fold increase in mRNA synthesis from this gene (Fagan et al., 1982). These DNase I hypersensitive sites are not found in rat thymus which does not synthesize cytochrome P-450M. Thus, the hypersensitive sites reflect the specific chromatin organization of the P-450M gene into a transcribable conformation, perhaps associated with the nuclear matrix. The presence of indistinguishable DNase I hypersensitive sites in both the induced and uninduced chromatin suggests that this gene is developmentally regulated similar to the β-globin gene in erythroblasts where the DNase I hypersensitive sites appear prior to gene expression, but only in cells destined to synthesize β-globin (Stalder et al., 1980).

Using a plasmid containing the entire cloned P-450M gene, we found that S1 nuclease preferentially cleaves the DNA sequence at a site very close to that which is cleaved in chromatin by DNase I. Similar findings were reported in the active β- and α-globin genes (Larsen & Weintraub, 1982; Schon et al., 1983; Cockerill & Goodwin, 1983; Nickol & Felsenfeld, 1983). Weintraub (1983) has interpreted these findings to mean that the DNA sequence recognizable by S1 may also be recognized by regulatory chromosomal proteins, ultimately resulting in an altered chromatin structure.

While the positions of the DNase I hypersensitive sites in the 5′ region of the P-450M genes are unaffected by gene induction, treatment of rats with 3-methylcholanthrene brings about a slight increase in the DNase I sensitivity of the P-450M gene as compared to that of a repetitive DNA sequence or an untranscribed gene such as insulin. The slight increase in DNase I sensitivity suggests an induction-sensitive change in chromatin structure to an active configuration similar to but much smaller than the large increase in DNase I sensitivity reported previously in *Drosophila melanogaster* hsp 70 genes following heat shock (Levy & Noll, 1981). Changes in DNase I sensitivity brought about by the transition from repressed to active gene expression are apparently not the same as the increased sensitivity reported in developmental systems (β-globin) or induced by hormones (ovalbumin and vitellogenin) [for a review, see Igo-Kemenes et al. (1982)]. In particular, the extent of the DNase I sensitive domains appears to be much smaller. For example, glyceraldehyde-3-phosphate dehydrogenase has a DNase I sensitive region extending 4 kb upstream of the 5′ end (Alevy et al., 1984). For ovalbumin, the DNase I sensitive region extends 7.8 kb 5′ from the transcribed sequences (Lawson et al., 1982). However, the DNase I sensitive region in heat-shocked *Drosophila melanogaster* includes only the coding regions of the hsp 70 gene (Levy & Noll, 1981).

The question arises as to what level of control brought about the change we detect in the chromatin conformation of the P-450M gene. Other rat liver genes appear to be developmentally regulated. Nahon et al. (1984) examined in DNase I sensitivity of the adult rat liver albumin and α-fetoprotein genes and found them identical even though the α-fetoprotein is not expressed in adult liver. The slight change in DNase I sensitivity of the cytochrome P-450M gene suggests that induction brings about a change in the chromatin conformation. However, the primary mechanism of regulation of gene expression at the chromatin level is developmentally determined because the DNase I hypersensitive sites which are present in the liver do not change upon gene activation.

Our present studies are the first studies on the chromatin structure of a member of the cytochrome P-450 family of enzymes. Analysis of other members of the cytochrome P-450 family chromatin structure will be required to determine if the pattern of chromatin modification following induction observed in P-450M is characteristic. If indeed it is characteristic of this family of genes that no new DNase I hypersensitive sites are generated upon induction, it could be proposed that this level of chromatin structure of the cytochrome P-450 genes is developmentally regulated.

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