Gene-Specific Differences in the Supranucleosomal Organization of Rat Liver Chromatin†

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ABSTRACT: Rat liver chromatin was separated into a solubilized portion and insoluble nuclear material, and the solubilized portion was fractionated by sucrose gradient sedimentation. The chromatin encompassing three transcribed genes (albumin, tryptophan oxygenase, and α-fetoprotein), which are expressed at very different levels, partitions preferentially with insoluble nuclear material and possesses a disrupted nucleosome structure. On the contrary, the chromatin encompassing three inactive genes fractionates into the solubilized chromatin portion and exhibits a canonical nucleosome repeat structure. By sucrose gradient sedimentation, all size classes of inactive chromatin particles are found to contain internal cleavages in the linker region between nucleosomes; they are probably held together by histone H1 and mono- and divalent cations. When the chromatin encompassing two flanking sequences of the tyrosine aminotransferase gene is studied, the 0.35-kilobase upstream-located chromatin exhibits features of active genes, while the 2.55-kilobase upstream-located chromatin partitions preferentially with solubilized chromatin and sediments in internally cleaved particles.

Gene expression in eucaryotes appears to be regulated at, at least, two different levels (Weintraub, 1985). A first more general mechanism operates on chromatin domains containing single genes or gene families. This mechanism does not regulate directly the level of transcription of a single gene but rather determines its potential to be transcribed in a, apparently, strictly yes-or-no mode. A second mechanism operates on genes derepressed at the first level and determines directly the rate of transcription of specific genes (rather than gene families). This second mechanism is probably mediated by trans-acting factors, and our knowledge on such factors is rapidly growing. On the other hand, the molecular basis of the first mechanism is poorly understood. It seems to be self-propagating, and the so-called "memory effect" of genes, which have a history of transcription and have the ability to be reactivated, is probably based on this self-propagating property (Alberts et al., 1977; Weintraub, 1985). Further, the first mechanism appears to be cis-acting and operative by determining the supranucleosomal structure of chromatin (Weintraub & Groudine, 1976; Weintraub, 1985).

Active chromatin differs from inactive chromatin in a wide variety of features including increased sensitivity to nuclease digestion, higher frequency of nuclease-hypersensitive sites, differences in solubility, lack of nucleosome repeat structure, altered supranucleosomal organization, and decreased level of DNA methylation. Also, changes in protein composition, such as histone acetylation, ubiquitination, and the presence of high mobility group proteins and certain histone variants, have been described. Employing various chromatin fractionation procedures, it has been further found that a great number of active genes partitioned with insoluble nuclear material under very different salt conditions (Jackson et al., 1981; Robinson et al., 1982; Ciejek et al., 1983; Jost & Seldran, 1984; Hentzen et al., 1984; Rose & Garrard, 1984; Strätling et al., 1986); on the contrary, inactive genes fractionated into the soluble chromatin portion. When circular DNA molecules were injected into oocytes, they assembled either into soluble minichromosomes, which exhibit a normal nucleosome repeat structure, or into insoluble ones lacking such a repeat structure (Ryoji & Worcel, 1985). It would be interesting to know whether cellular bulk chromatin also falls into two strictly separated classes. As a first approach to this question, the solubility and repeat structure of chromatin encompassing murine immunoglobulin light-chain genes and the chicken lysozyme gene have been investigated (Rose & Garrard, 1984; Strätling et al., 1986). The present paper extends these studies to rat liver chromatin encompassing various active and inactive genes and two flanking sequences of an actively transcribed gene. From the class of genes active in rat liver, I selected three, which extremely diverge in their level of being expressed: albumin, tryptophan oxygenase, and α-fetoprotein. The albumin gene belongs to the most frequently transcribed genes in the adult animal, while the tryptophan oxygenase gene appears to be transcribed more rarely, even following hormonal induction (Killewich et al., 1975). The α-fetoprotein gene is apparently transcribed at an extremely low level in the adult rat but can be activated severalfold during carcinogenesis or liver regeneration (Petropoulos et al., 1983; Sala-Trepat et al., 1979). As examples of the class of sequences residing in the 5′-flanking region of active genes, I selected two single-copy sequences located between 2.95 and 2.55 kilobases (kb)[1] and between 0.8 and 0.35 kb, respectively, upstream of the transcription start site of the tyrosine aminotransferase gene. The 5′-flanking region of the tyrosine aminotransferase gene contains several hypersensitive sites: The first one is located at the promoter, a second one at about 1 kb upstream of the transcription start site, and a third one, which is induced following induction by glucocorticoids, at 2.5 kb upstream (Becker et al., 1984, 1986). Thus, the sequences selected are located near these hypersensitive sites but do not overlap with these. From the class of genes inactive in rat liver, the genes for oxytocin, vasopressin, and prolactin were studied. In the

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[1] Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; kb, kilobase(s); EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; bp, base pair(s).

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studies presented here, active chromatin was found to partition with insoluble nuclear material and to possess a disrupted nucleosome structure. On the contrary, inactive chromatin partitions with the solubilized chromatin portion, exhibits a normal nucleosome repeat structure, and sediments in particles which contain internal cleavages but are held together probably by histone H1 and mono- and divalent cations.

Materials and Methods

Males rats (Wistar) received intraperitoneal injections of 5 mg/100 g body weight of hydrocortisone (Upjohn) and were sacrificed 2.5 h later. Liver nuclei were prepared as described previously (Strätling & Klingholz, 1981) except that purified nuclei were resuspended in 0.1 M sucrose, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.14 mM spermidine, and 10 mM Tris-HCl, pH 7.5. Nuclei were digested for the indicated times at 37 °C using the endogenous Ca/Mg-dependent endonuclease and then chilled on ice. After removal of one-tenth of the suspension (unfractionated control), nuclei were pelleted by centrifugation at 5000 rpm for 10 min in an HB4 rotor of the Sorvall centrifuge; the supernatant (chromatin fraction S1; 1.8% of the total nuclear DNA) was saved. The pelleted samples were resuspended in 5 mM KCl, 0.4 mM $MgCl_2$, 2 mM EGTA, and 5 mM Tris-HCl, pH 7.5, and incubated for 1 h on ice. The supernatant was then centrifuged at 10 000 rpm for 10 min to generate a second supernatant fraction, S2, containing solubilized chromatin and a pelleted fraction, P2, containing insoluble nuclear material; fraction P2 included 29.5% ± 4.1% of total nuclear DNA (mean ± SE of four experiments). Aliquots of the solubilized chromatin (200 μL in analytical gradients and 500 μL in preparative gradients) were layered on isokinetic sucrose gradients in 5 mM KCl, 0.5 mM $MgCl_2$, 0.1 mM EGTA, and 5 mM Tris-HCl, pH 7.5, and centrifuged in a Beckman SW40 rotor at 40 000 rpm and 4 °C for 2 h (Strätling & Klingholz, 1981). Gradients were fractionated and monitored for the absorbance at 254 nm as described previously (Strätling & Klingholz, 1981). Corresponding fractions from preparative sucrose gradients were pooled, dialyzed first against 0.5 mM EDTA and 5 mM Tris-HCl, pH 7.5, and then against distilled H_2O to remove sucrose, and lyophilized. Purification of DNA from each fraction and electrophoresis in 1.25% agarose gels were carried out as described (Strätling & Klingholz, 1981). Histones were extracted with 0.4 N H_2SO_4, displayed by electrophoresis on a 15% (w/v) polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate (Laemmli, 1970), and visualized by staining with Amido Black 10B.

The relative abundance of specific sequences was determined by dot-blotting of the purified DNA onto nitrocellulose filters and hybridization with ^{32}P-labeled nick-translated probes followed by autoradiography (Strätling et al., 1986). Southern-blotting and hybridization was performed as described previously (Strätling et al., 1986).

The molecular probes used are as follows. pALB is a cDNA albumin clone containing a 0.8-kb insert constructed by Dr. W. Roewekamp. pUTO EX2.7 is a genomic clone of the tryptophan oxygenase gene containing 2.7 kb of single-copy coding sequences (including exons E and F) (Schmid et al., 1982). pBR-cAFP is a cDNA α-fetoprotein clone (Innis et al., 1979). pSPT PX0.4 contains a 0.4-kb single-copy fragment located between 2.95 and 2.55 kb upstream of the transcription start site of the tyrosine aminotransferase gene, and PA 450 contains a 0.45-kb single-copy fragment located between 0.8 and 0.35 kb upstream of the transcription start site of the same gene (Becker et al., 1984). pUC OT-3′ and pUC VP-3′ are genomic clones containing a 0.7-kb fragment of the oxytocin gene and a 0.64-kb fragment of the vasopressin gene, respectively (Ivell & Richter, 1984); both fragments do not cross-hybridize to each other (Ivell & Richter, 1984). pPRL-1 is a cDNA prolactin clone (Gubbins et al., 1979).

Results

Active Genes Are Preferentially Insoluble. In order to study the chromatin organization of specific genes in rat liver nuclei, the present investigation utilized digestion with the endogenous Ca/Mg-dependent endonuclease and chromatin fractionation. In the fractionation course, digested nuclei were pelleted to remove the small amounts of chromatin released during digestion (fraction S1) and resuspended in 5 mM K^+/0.4 mM $MgCl_2$/5 mM Tris-HCl, pH 7.5. The solubilized chromatin portion (fraction S2) was separated by centrifugation from insoluble nuclear material (fraction P2) and size-fractionated by sedimentation in isokinetic sucrose gradients. This method was modified from protocols published previously (Strätling & Klingholz, 1981; Klingholz & Strätling, 1982) by reducing the monovalent cation concentration to 5 mM K^+ in order to slow the exchange of H1 (Thomas & Rees, 1983) and by speeding up chromatin solubilization by resuspending pelleted nuclei rather than by overnight dialysis. When nuclei were digested for 8–22 min, the sedimentation profiles are bimodal, exhibiting a peak containing mononucleosomes and short-chain oligonucleosomes and a broad peak containing larger size chromatin particles (Figure 1). In order to determine quantitatively the partitioning of three active genes, three inactive ones, and two flanking sequences during chromatin fractionation, liver nuclei from steroid-induced rats were autodigested for 16 min, and chromatin was fractionated as above but using a preparative sucrose gradient (Figure 2A). Eight sets of 2 μg of purified DNA from the unfractionated digest (U), from chromatin fraction S1, from the insoluble nuclear material (P2), and from each gradient fraction were dot-blotted onto nitrocellulose filters, and each set was hybridized to a specific ^{32}P-labeled nick-translated probe followed by autoradiography (Figure 2B). The partitioning of a particular sequence between the solubilized chromatin fraction and the insoluble chromatin portion was estimated by comparing the signal exhibited by hybridization of DNA from the insoluble material with a labeled probe for that sequence to the signal exhibited by hybridization of DNA from the unfractionated digest to the same probe. Taking further into account that the insoluble portion contains 29.5% of total nuclear DNA (see Materials and Methods), approximately 98% of the albumin-coding sequences partition with insoluble nuclear material. Consequently the abundance of the albumin gene is significantly depleted in all size classes of chromatin particles separated by sucrose gradient sedimentation as verified in Figure 2B. Approximately 41% of the *Eco*RI–*Xho*I fragment containing exons E and F and abutting intervening sequences of the tryptophan oxygenase gene (Schmid et al., 1982) and approximately 50% of the α-fetoprotein gene partition—in repeated experiments—with insoluble nuclear material. Thus, although the α-fetoprotein gene appears to be transcribed at a level approximately 16-fold lower than that of the tryptophan oxygenase gene [Killewich et al., 1975; Petropoulos et al., 1983; see also Discussion], the partition response of both genes during chromatin fractionation is very similar. Further, the partition response of the tryptophan oxygenase gene in liver chromatin from non-steroid-induced rats is nearly identical with that in chromatin from glucocorticoid-induced rats (not shown) (Schütz et al., 1975). It has to be concluded that transcribed sequences partition preferentially with insoluble nuclear material but that the extent of this partitioning does not correlate

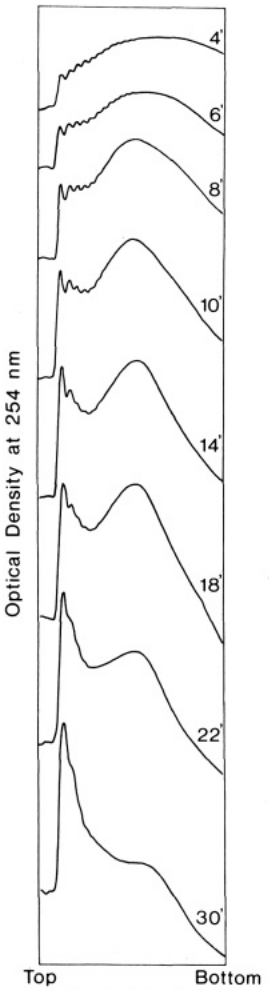

FIGURE 1: Sedimentation of solubilized rat liver chromatin in a time course experiment. Rat liver nuclei were digested with the endogenous endonuclease for the indicated periods of time (minutes), and the solubilized chromatin was sedimented (from left to right) in isokinetic sucrose gradients. The sedimentation curves are arbitrarily positioned on the ordinate in order to facilitate comparison.

in a simple way to the rate of expression of the respective gene.

The fourth and the fifth rows in Figure 2B show the partition response of the two single-copy sequences selected from the 5′-flanking region of the tyrosine aminotransferase gene. Approximately 63% of the upstream-located sequence (2.55-TAT) and approximately 34% of the downstream-located one (0.35-TAT) fractionate into the solubilized chromatin portion (S2), while the remainder is recovered with insoluble nuclear material. The last three rows in Figure 2B show that about 86% of the oxytocin gene, 82% of the vasopressin gene, and 72% of the prolactin gene partition with solubilized chromatin (S2) during chromatin fractionation. Thus, inactive genes fractionate preferentially into the soluble chromatin portion, and sequences located at some distance from active genes appear to respond in a similar way. However, noteworthy, the upstream-located tyrosine aminotransferase sequence (2.55-TAT) is distributed slightly differently across the sucrose gradient than inactive genes: The abundance of the three studied inactive genes increases gradually from gradient fraction 3 to fraction 8 as seen most clearly with the prolactin probe, while the abundance of the tyrosine aminotransferase sequence is relatively invariant in these fractions. A caveat concerns gradient fraction 1: The hybridization signal exhibited by DNA prepared from this fraction is relatively weak compared to that of fractions 2 and 3. As fraction 1 contains mainly mononucleosomal DNA fragments (see Figure 2C),

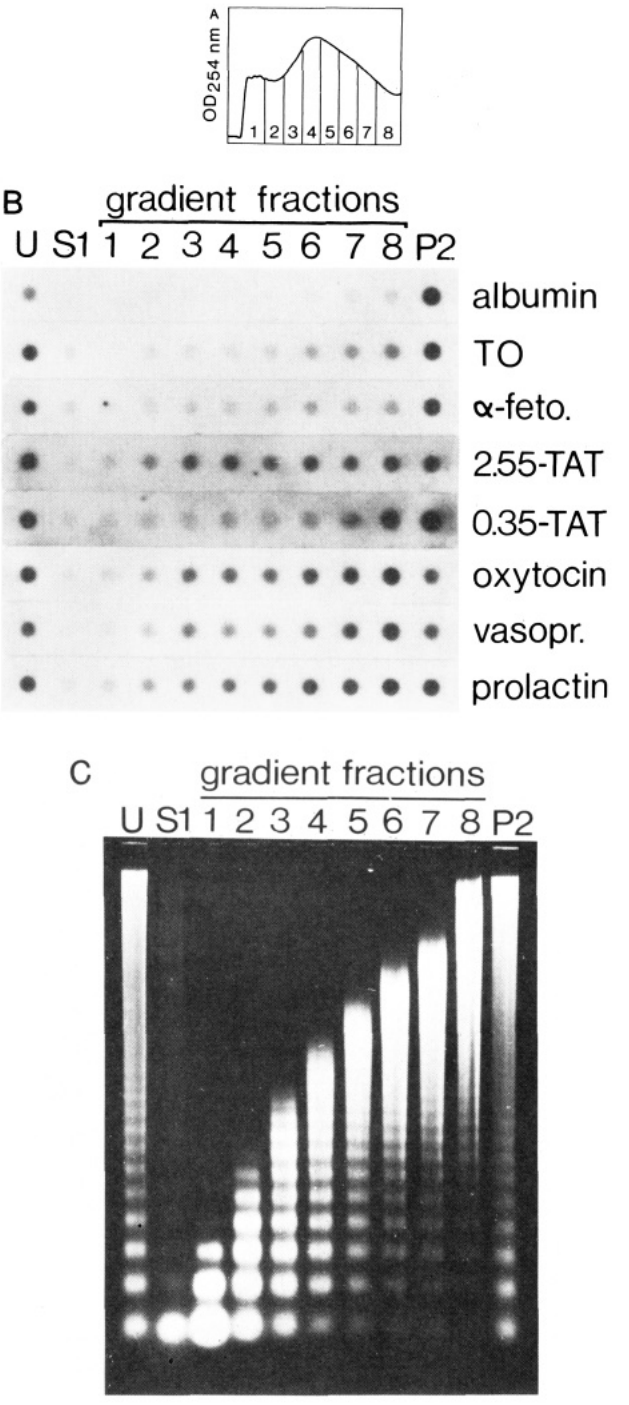

FIGURE 2: Partitioning of active and inactive sequences during fractionation of rat liver chromatin. Following endogenous digestion of rat liver nuclei for 16 min, chromatin was fractionated into a supernatant fraction (S1), a solubilized chromatin portion (S2), and insoluble nuclear material (P2). 500-μL aliquots of the solubilized chromatin fraction were then sedimented on preparative sucrose gradients. The gradients were separated into eight fractions (1–8 from top to bottom) as shown in (A), and equivalent fractions were pooled. Fraction 8 includes the material pelleted onto the bottom of the SW40 tube. (B) Eight sets of 2 μg each of purified DNA of each gradient fraction, of the unfractionated digest (U), and of chromatin fractions S1 and P2 were dot blotted onto nitrocellulose filters, and the filters were hybridized with a ^{32}P-labeled nick-translated albumin gene probe, a tryptophan oxygenase gene probe (TO), an α-fetoprotein gene probe (α-feto.), a probe located 2.55 kb upstream of the tyrosine aminotransferase gene (2.55-TAT), a probe located 0.35 kb upstream of the same gene (0.35-TAT), an oxytocin gene probe, a vasopressin gene probe (vasopr.), and a prolactin gene probe, respectively. (C) Distribution of DNA fragment sizes. Five micrograms of the fractions analyzed in (B) was electrophoresed on a 1.25% agarose gel and stained with ethidium bromide.

it is possible that nonquantitative binding of the smallest fragments to the filter in the dot-blotting procedure contributes, artifactually to the relatively weak hybridization signal of gradient fraction 1 DNA.

Soluble Chromatin Particles Encompassing Inactive Genes Exhibit a Canonical Nucleosome Structure with Internal Cuts, While Insoluble Chromatin Encompassing Active Genes Shows a Disrupted Nucleosomal Organization. The length distribution of DNA fragments in each chromatin fraction of the preparative sucrose gradient in Figure 2C exhibits two main features. The upper size limit of the DNA fragments increases continuously with the sedimentation rate of the respective chromatin particles. Second, each size class of chromatin particles exhibits a ladder of nucleosomal DNA fragments, which extends down to monomer-length fragments. Thus, the length distribution of continuous arrays of nucleosomes in each chromatin size fraction ranges from mononucleosomes up to uncleaved particles. The insoluble nuclear material (P2) comprises DNA fragments which range from mononucleosomal fragments up to the largest DNA pieces found in the digest. Three control experiments indicate that the content of mononucleosomes and short-chain oligonucleosomes in large-size chromatin particles does not result from artifactual aggregation on the gradient (not shown). First, in a concentration series experiment, the length distribution of DNA fragments in each chromatin fraction does not change, when lower amounts of fraction S2 (up to one-fifth) are loaded onto the gradient. Second, in a digestion time course experiment, the distribution of DNA fragments in a given chromatin fraction shifts to smaller fragments with increasing digestion. Third, in a cation concentration experiment, the DNA fragment distribution does not change within a range extending from 10 mM NaCl plus KCl/0.2 mM $MgCl_2$ to 50 mM NaCl plus KCl/1 mM $MgCl_2$. It has to be concluded that the solubilized chromatin particles in each S2 size fraction are held together despite internal cleavages in the linker regions between nucleosomes. The P2 DNA size distribution also exhibits a regular nucleosomal ladder; however, due to an increase in background DNA between the bands, the P2 DNA ladder is more "smeary" than that of the unfractionated digest and of any of the sucrose gradient fractions. As will be shown later in Figure 4, transcribed genes are cleaved by the endogenous nuclease into a continuum of fragment sizes. Since transcribed genes are enriched in fraction P2 (see Figure 2B), it follows that the smeary feature of the P2 DNA nucleosomal ladder arises most likely from the elevated abundance of transcribed sequences.

In order to investigate the DNA fragment size distribution in chromatin particles harboring specific sequences, four sets of DNA samples from gradient fractions 1–8 were electrophoretically resolved and transferred to nitrocellulose filters for hybridization with ^{32}P-labeled inactive gene probes and the 2.55-kb upstream-located tyrosine aminotransferase probe, respectively. Since the three inactive gene probes produced identical autoradiograms, only the results for the oxytocin gene are shown. Figure 3A first demonstrates that the intensity of the hybridization signal increases from gradient fraction 3 to fraction 8, thus reproducing the result in Figure 2B. Second, in each size class of chromatin particles, the oxytocin gene fragments display a normal nucleosomal ladder and exhibit a size distribution which is similar to that of bulk DNA (see the ethidium bromide stained gel in Figure 2C). The only variance found is that monomer-length fragments exhibit a relative hybridization signal which is weak in comparison to the relative amount of DNA in these fragments, probably

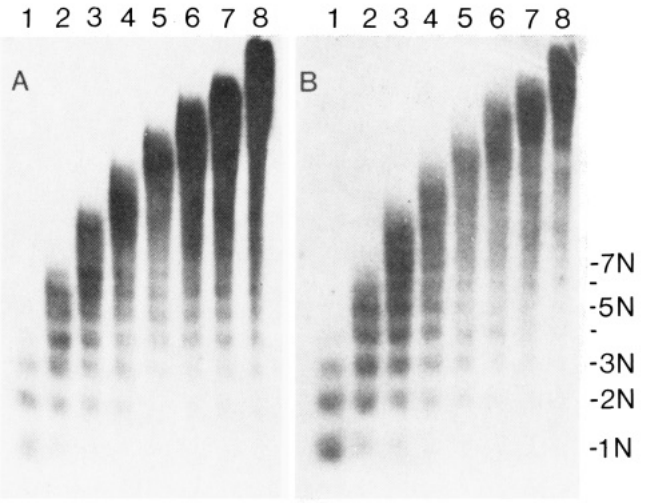

FIGURE 3: Internal cleavages in chromatin particles encompassing inactive genes and encompassing a sequence located 2.55 kb upstream of the tyrosine aminotransferase gene. Two sets of DNA samples of gradient fractions 1–8 shown in (A) were electrophoreticaly resolved and transferred onto nitrocellulose filters for hybridization with ^{32}P-labeled nick-translated probes of the oxytocin gene (A) and of a 2.55 kb upstream-located tyrosine aminotransferase sequence (B), respectively, 1N, 2N, etc. denote the position of monomeric, dimeric, etc. nucleosomal DNA fragments.

because they bound less efficiently to the filter than larger fragments. The results thus show that—in three specific cases—chromatin particles packaging inactive genes remain intact despite internal cleavages in the linker regions between nucleosomes.

The fourth Southern-blot filter was hybridized to the 0.4-kb probe located 2.55-kb upstream of the tyrosine aminotransferase gene (Figure 3B). The lanes analyzing gradient fractions 3–8 exhibit total hybridization signals which are similar in intensity, confirming the above result of a nearly invariant abundance of the flanking tyrosine aminotransferase sequence in these fractions (see Figure 2B). Within each chromatin size fraction, the DNA fragments show a regular nucleosomal ladder, which by linear regression analysis possesses an average-repeat length identical with that of the oxytocin gene. However, a comparison of corresponding lanes in Figure 3A and Figure 3B reveals that, in chromatin particles of the same size class, the distribution of flanking tyrosine aminotransferase gene fragments is shifted to shorter sizes relative to the distribution of oxytocin gene fragments. This is most clearly seen in that portion of the autoradiogram analyzing gradient fractions 4 and 5: The lanes of tyrosine aminotransferase fragments are more "tailing" in appearance than those of oxytocin fragments. It is concluded that approximately 63% of the chromatin encompassing the 2.55-kb upstream-located tyrosine aminotransferase sequence possesses a normal nucleosome repeat structure and that it sediments in particles, that are held together despite numerous internal cleavages.

In contrast to the canonical nucleosomal ladder of fragments exhibited by inactive genes, the transcribed genes for albumin and tryptophan oxygenase (and the α-fetoprotein gene; not shown) are cleaved into a nondiscrete continuum of fragment sizes as shown by hybridization of the electrophoretically resolved and blotted DNA fragments of chromatin fraction P2 (Figure 4). This demonstrates that these transcribed genes possess a disrupted nucleosome structure. Interestingly, that portion of the chromatin encompassing the 2.55-kb upstream-located tyrosine aminotransferase sequence, which was found in insoluble nuclear material, was also cleaved into a

FIGURE 4: Disrupted nucleosome structure in chromatin encompassing active genes. Three sets of DNA samples of the insoluble chromatin fraction P2 were electrophoretically resolved and transferred onto nitrocellulose filters for hybridization with ^{32}P-labeled nick-translated probes for the albumin gene, the tryptophan oxygenase gene (TO), and the 2.55 kb upstream-located tyrosine aminotransferase sequence (2.55-TAT), respectively. The arrowhead indicates the position of mononucleosomal DNA.

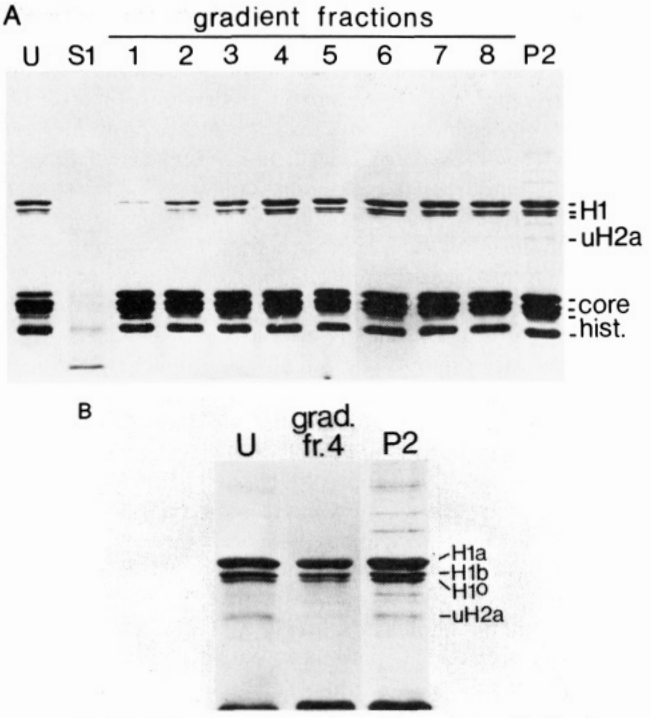

FIGURE 5: Protein composition of rat liver chromatin fractions. Following endogenous digestion of rat liver nuclei, chromatin was fractionated by solubilization and sedimentation in the same way as in the experiment shown in Figure 2. (A) Basic proteins were extracted with 0.4 N H_2SO_4 from the unfractionated digest (U), from chromatin fractions S1 and P2, and from each sucrose gradient fraction and displayed by electrophoresis on a 15% polyacrylamide gel in the presence of sodium dodecyl sulfate. (B) Enlarged view of the H1–uH2a region of three selected lanes in (A): U, unfractionated digest; grad. fr. 4, gradient fraction 4; uH2a, ubiquitinated H2a.

continuum of DNA fragment sizes Figure 4). Thus, the fractionation procedure partitions the flanking tyrosine aminotransferase chromatin into two distinct portions, which are very different with respect to their solubilities and supranucleosomal organizations.

Histone H1 Content. The basic protein composition is displayed by electrophoresis on a 15% polyacrylamide gel in the presence of sodium dodecyl sulfate (Figure 5). The chromatin particles in all sucrose gradient fractions and the insoluble nuclear material contain all four core histones at approximately equimolar levels. The content of histone H1 relative to the core histones was determined by scanning the gel in Figure 5A. In gradient fractions 1 and 2, it is reduced to 24% and 69%, respectively, of that in the unfractionated digest; it is nearly identical with that in the unfractionated digest in fractions 3 and 4 and slightly increased in fractions 5–8 (approximately 1.23-, 1.21-, 1.22-, and 1.23-fold, respectively) (Figure 5A). It is also increased (approximately 1.39-fold) in the insoluble nuclear material (fraction P2). Rat liver chromatin contains, on the average, 0.8 mol of H1 per core histone octamer as determined chemically by Bates and Thomas (1981). When this value is applied to the unfractionated digest in Figure 5A, fractions 1–8 were calculated to contain the following moles of H1 per core histone octamer: 0.20, 0.55, 0.78, 0.83, 0.98, 0.97, 0.98, and 0.98, respectively. Fraction P2 was found to contain 0.83 mol of H1 per core histone octamer (mean of four experiments). These results appear to indicate that a limited rearrangement of H1 occurred from mononucleosomes (and perhaps short-chain oligonucleosomes) to larger size particles. If rat liver chromatin was fractionated at 60 mM NaCl, a much more pronounced rearrangement of H1 was found within the solubilized chromatin portion, generating, e.g., an increase in the molar H1 content in the fastest sedimenting particles (fraction 8) up to 1.49 mol (not shown). Thus, H1 rearrangement depends on the ionic conditions, being significantly more promoted at 60 mM NaCl/0 $MgCl_2$ than at 5 mM KCl/0.4 mM $MgCl_2$. The fractionation experiment at 60 mM NaCl further shows that, up to a stoichiometry of 1.49 mol, chromatin particle solubility is not limited by H1. In all chromatin fractions from the sucrose gradient, the band containing ubiquitinated H2a is less intense than that in the unfractionated digest, and, conversely, in the insoluble chromatin fraction, it is slightly more intense (Figure 5B). As transcribed sequences partition preferentially with insoluble nuclear material (see Figure 2), this finding is consistent with previous propositions that ubiquitinated H2a is preferentially localized in the active chromatin portion (Goldknopf et al., 1980; Levinger & Varshavsky, 1982). The staining intensity of the H1° band relative to that of the H1a and H1b bands is further found to be elevated in the insoluble chromatin fraction when compared to the unfractionated digest; this may result from a minor differential rearrangement of H1 variants during digestion or chromatin fractionation rather than be related to a nonrandom location of H1° (Roche et al., 1985; Delabar, 1985).

Discussion

Rat liver chromatin was separated into a solubilized portion and insoluble nuclear material, and the chromatin particles in the solubilized portion were size-fractionated by sucrose gradient sedimentation. Three transcribed genes, which are expressed at very different levels (albumin, tryptophan oxygenase, and α-fetoprotein), are found to partition preferentially with insoluble nuclear material. Very related results were described recently for the lysozyme gene in hen oviduct chromatin (Strätling et al., 1986) and for various other actively transcribed genes (Jackson et al., 1981; Robinson et al., 1982; Ciejek et al., 1983; Hentzen et al., 1984). The biochemical

basis for the specific partitioning of active chromatin with insoluble nuclear material is not known. Either a component of active chromatin mediates attachment to an insoluble nuclear structure or active chromatin coaslesces by the induction of hydrophobic interactions during nuclei or chromatin preparation (Ryoji & Worcel, 1985). In liver hepatocytes, the albumin gene belongs to the class of genes which are transcribed at a very high frequency. The tryptophan oxygenase gene, on the other hand, appears to be transcribed more rarely, since in a cell-free system using polyadenylated liver mRNA of induced rats, tryptophan oxygenase synthesis represents only about 0.1% of total protein synthesis (Killewich et al., 1975). However, as tryptophan oxygenase is subject to hormonal regulation, and as its mRNA therefore is likely to have a shorter half-life than albumin mRNA, the relative rate at which the tryptophan oxygenase gene is transcribed may be higher than would be expected from the mRNA abundance. The α-fetoprotein gene, thirdly, appears to be transcribed only scarcely as the respective mRNA constitutes approximately 0.006% of the polysomal polyadenylated liver RNA (Petropoulos et al., 1983). As approximately 98%, 41%, and 50% of the albumin, tryptophan oxygenase, and α-fetoprotein gene, respectively, fractionate into the insoluble nuclear portion, the extent of partitioning with insoluble material does not seem to correlate in a simple way to the relative degree of expression of these genes. That the α-fetoprotein gene, which is apparently expressed about 16-fold less than the tryptophan oxygenase gene, exhibits a similar partition response as the latter gene is perhaps most surprising. As an explanation, the genomic location of the α-fetoprotein gene is in close proximity to the very active albumin gene, and, furthermore, the harboring of both genes in a common domain or loop may confer to α-fetoprotein chromatin features of very active chromatin (Chevrette et al., 1987). Contrary to transcribed genes, three inactive genes (oxytocin, vasopressin, and prolactin) are found in the solubilized chromatin portion.

Following fractionation of the solubilized chromatin portion by sucrose gradient sedimentation, all size classes of bulk chromatin particles are shown to contain internal cleavages in the linker region between nucleosomes. Previous studies demonstrated that they are held together by the peripheral histone H1 and by mono- and divalent cations (Thoma et al., 1979; Strätling & Klingholz, 1981). The nearly invariant position of the top of the supranucleosomal particle peak in digestion time course experiments had suggested to Strätling and Klingholz (1981) that a distinct size class of particles exhibits a preferred metastability within a critical range of mono- and divalent cations (between 10 mM Na^+ plus K^+/0.2 mM Mg^{2+} and 50 mM Na^+ plus K^+/1 mM Mg^{2+}). Whether these particles arise by aggregation during fractionation (Muyldermans et al., 1985) or by disassembly at periodically spaced, "weak" sites along the 30-nm fiber (Strätling & Klingholz, 1981; Walker et al., 1986) is not clear. Alternatively, the invariant position of the supranucleosomal particle peak may be produced by a molecular weight dependent decrease of the solubility of particles larger than 15–20 nucleosomes. It should be emphasized, however, that this controverse discussion does not disturb the present study on the structure of chromatin encompassing various rat liver genes.

By Southern-blot analysis, it is shown that chromatin encompassing inactive genes possesses a canonical nucleosome repeat structure and that size-fractionated inactive chromatin particles contain internal cleavages in the linker regions approximately as frequently as bulk chromatin. While the experiments here do not establish a direct proof, it is likely that inactive chromatin particles—in analogy to bulk chromatin particles—are held together by H1 and mono- and divalent cations. In contrast to inactive genes, transcribed genes—found in the insoluble nuclear fraction—are cleaved into a nondiscrete continuum of DNA fragment sizes establishing a disrupted nucleosome structure. The differences in solubility between active and inactive chromatin might be a reflection either of the differences in supranucleosomal organization or of the presence of a specific protein component in active chromatin. As the present paper shows that packaging into internally cleaved chromatin particles probably held together by H1 is a specific feature of inactive chromatin, it adds support to the notion that folding into this structure is part of the self-propagating mechanism regulating the expression of domains of genes or gene families (Weintraub, 1985; see also the introduction). The property of H1 to repress gene activity has also been demonstrated directly in an in vitro transcription system using the oocyte 5S RNA genes of *Xenopus laevis*, which are inactive in somatic cells (Schlissel & Brown, 1984). After removal of H1, these genes are transcribed, and adding H1 back reestablishes their repression. However, binding of transcription factors prior to H1 addition does not regenerate the native repressed chromatin structure.

The mononucleosomal and short-chain oligonucleosomal fraction was found to be depleted in histone H1, while—relative to the average stoichiometry in nuclei—the fastest sedimenting particles (gradient fractions 6–8) exhibit a slightly increased molar ratio of H1. Apparently, digestion at the utilized ionic conditions produced a limited rearrangement of H1. Interestingly, 60 mM NaCl/0 $MgCl_2$ promotes this rearrangement significantly more than 5 mM KCl/0.4 mM $MgCl_2$. Determining the exchange of H1 between previously separated big and little chromatin fragments, Jin and Cole (1986) observed a relative exchange of 10% in 0.5 mM $MgCl_2$, while Caron and Thomas (1981) reported an exchange from 50% to 100% at 30–70 mM NaCl. If the assay conditions in both cited reported are comparable, the relative effects of the compared ionic conditions on the exchange of H1 would be qualitatively similar to those on H1 rearrangement. The mononucleosomes in the low molecular weight peak contain, on the average, 166 bp DNA fragments, while the mononucleosomes in the larger chromatin particles contain DNA fragments approximately 170–200 bp in length (Klingholz & Strätling, 1982). The nibbling-off of the linker region by the endonuclease and, thus, the removal of an important portion of the binding site of H1, are, probably, the basis for the rearrangement of H1 to larger chromatin particles, in particular since nucleosomes have been reported to contain two binding sites for H1 (Nelson et al., 1979).

Nuclease digestion and chromatin fractionation was also used to characterize two selected chromatin regions encompassing sequences on the 5′ side of the tyrosine aminotransferase gene. Approximately 63% of the DNA fragments located between 2.95 and 2.55 kb upstream of the gene partition with the solubilized chromatin portion, while about 66% of the fragments located between 0.8 and 0.35 kb are recovered with insoluble nuclear material. The solubilized 2.55-kb upstream chromatin exhibits a normal nucleosome repeat structure. The chromatin particles derived from it contain internal cleavages in the linker region but at a higher frequency than chromatin packaging inactive genes. Further, the distribution of the 2.55-kb upstream sequence across the sucrose gradient in Figure 2A is shifted slightly to smaller chromatin particles when compared to the distribution of inactive genes across the same gradient. Both features are explained most

easily by an elevated sensitivity of this chromatin portion to nuclease digestion. A preferred cutting at the hypersensitive site located closely downstream of the probing fragment may further contribute to the described shift in the distribution of the 2.55-kb upstream sequence across the gradient (Becker et al., 1984, 1986). Interestingly, the insoluble portion of the 2.55-kb upstream tyrosine aminotransferase chromatin exhibits a disrupted nucleosome structure. This would support the notion that the differences in chromatin solubility might reflect differences in nucleosomal and supranucleosomal structure.

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