The mouse albumin promoter and a distal upstream site are simultaneously DNase I hypersensitive in liver chromatin and bind similar liver-abundant factors in vitro

Jen-Kuei Liu, Yehudit Bergman,¹ and Kenneth S. Zaret²

Section of Biochemistry, Brown University, Providence, Rhode Island 02912 USA

In this paper we characterize the chromatin structure and nuclear proteins associated with different transcriptional states of the mouse serum albumin gene. We found the albumin gene to be transcribed in liver at rates 1000-fold or greater than in other tissues tested. We discovered seven DNase I hypersensitive sites encompassing the albumin gene only in liver chromatin, with strong hypersensitivity at the promoter and the enhancer, which is over 10 kb upstream. Using a gel retardation assay, we found a liver nuclear protein, or set of proteins, which binds specifically to DNA of a liver-specific hypersensitive site that maps 3.5 kb upstream, between the promoter and enhancer. Footprinting, heat insensitivity, and binding competition experiments indicate that the protein(s) have characteristics similar to a heat-stable, liver-abundant protein that binds to the albumin promoter and other enhancer and promoter sequences. Finally, we asked whether the liver-specific factors that cause DNase I hypersensitivity in vivo are present concurrently at the various sites in chromatin. We devised a simple new method to reveal that in liver, individual albumin genes are hypersensitive simultaneously at the promoter, the enhancer, and the -3.5-kb site. Thus, transcriptionally active albumin genes appear to contain tissue-abundant factors that are present at three widely spaced points in chromatin, yet at the same point in time. Similar factors binding simultaneously to at least two of these sites could create a specific structure in chromatin required for high-level albumin gene transcription.

[**Key Words:** Tissue-specific transcription; chromatin hypersensitive sites; mouse albumin gene; DNA-binding proteins]

Received July 8, 1987; revised version accepted March 18, 1988.

We are interested in factors that cause the serum albumin gene to be highly transcribed in only the liver of adult mice, as a model system for studying cell-specific gene control. In the mouse, the appearance of albumin gene transcription is coincident with the development of the fetal liver (Tilghman and Belayew 1982; Powell et al. 1984). Thereafter, the albumin gene is expressed constitutively in hepatocytes without additional forms of regulation that are observed for other liver-specific genes, such as transferrin (McKnight et al. 1980) or the evolutionarily related α -fetoprotein (Tilghman and Belayew 1982; Pachnis et al. 1984).

In this study, we first used the enzyme DNase I as a probe for chromatin features that correlate with tissue-specific transcription of the albumin gene. Many tran-

scribed genes contain sites in chromatin that are hypersensitive to DNase I (Stalder et al. 1980; Wu 1980); such sites often encompass DNA sequences that appear to be nucleosome-free in vivo and important for gene control (reviewed by Eissenberg et al. 1985). In addition, some hypersensitive sites appear to be due to nuclease affinity for inherent DNA structures that are prominent in both protein-free DNA and chromatin (Larsen and Weintraub 1982; Nickol and Felsenfeld 1983; Schon et al. 1983). Most likely, DNase I hypersensitivity arises because either DNA structure or specific binding of protein to DNA causes the stable loss of a nucleosome (McGhee et al. 1981; Ben Ezra et al. 1986); then DNase I has access to free DNA in the immediate vicinity of either the DNA structure or bound protein (Emerson and Felsenfeld 1984; Jackson and Felsenfeld 1985) in chromatin.

Because many genes possess DNase I hypersensitive sites only when the gene is transcriptionally active, and because some of these sites include known regulatory sequences (Wu 1980; Parslow and Granner 1982; Sher-

¹Present address: Hubert Humphrey Center for Experimental Medicine and Cancer Research, The Hebrew University, Hadassah Medical School, Jerusalem 91010 Israel.

²Corresponding author.

moen and Beckendorf 1982; Jongstra et al. 1984; Zaret and Yamamoto 1984), we used hypersensitivity as an assay to discover elements that putatively control albumin transcription. First, we quantitated the differential transcription rate of the albumin gene in three mouse tissues: liver, kidney, and spleen. Next, we found that each of the tissue-specific transcription levels was associated with a distinct pattern of DNase I hypersensitive sites in chromatin; in liver, we found the albumin locus to contain seven unique sites separated by up to 34 kb. Furthermore, we discovered protein(s) that bind in vitro to DNA of one of the liver-specific hypersensitive sites and compared characteristics of the binding factor(s) to proteins that are relevant to albumin promoter activity.

Like albumin, the globin (Stalder et al. 1980), vitellogenin (Burch and Weintraub 1983), and lysozyme (Fritton et al. 1984) genes have multiple hypersensitive sites in tissues where they are highly transcribed. However, hypersensitive sites of the other genes were mapped with indirect end-label probes (Wu 1980) in a manner that could not distinguish whether a given set of sites is hypersensitive on a single gene in a cell or whether factors causing hypersensitivity at different sites are present in different cells of the tissue. From a mechanistic point of view, it is important to know whether factors that cause hypersensitivity at different regulatory sites are present together temporally in chromatin. In this study, we asked which of the multiple sites, if any, are hypersensitive at the same time on a given albumin gene copy. The results are consistent with certain models for the role of distal regulatory elements in the maintenance of a transcriptionally active promoter.

Results

Differential transcription of the albumin gene

We determined rates of transcription of the albumin gene in different mouse tissues so we could compare albumin promoter activity to tissue-specific features of chromatin. We sacrificed a BALB/c mouse, isolated nuclei from liver, kidney, and spleen and performed run-on assays (Clayton et al. 1985a), in the presence or absence of 1 μ g/ml α -amanitin, to radioactively label RNA of preexisting nuclear transcription complexes. We hybridized the resulting 32 P-labeled RNAs to cloned mouse albumin cDNA (Kioussis et al. 1981) and other cDNA samples immobilized on nitrocellulose. RNase-resistant hybrids were visualized by autoradiography, as shown in Figure 1.

As expected, the data reveal that RNA polymerase II transcribes the albumin (Tilghman and Belayew 1982; Powell et al. 1984) and tyrosine aminotransferase (Scherer et al. 1982) genes at a high rate in liver. We detect slight transcription of albumin in the kidney in this and other hybridizations (data not shown) and no significant albumin transcription in the spleen. We quantitated the rate of transcription of the albumin gene in each tissue relative to ribosomal RNA (rRNA) gene tran-

Figure 1. Tissue-specific transcription of the albumin gene. Run-on transcription assays were performed in the presence of [32 P]UTP with nuclei from a mouse liver, kidney, and spleen in the presence (+) or absence (-) of 1 μ g/ml α -amanitin, as shown. We hybridized 4.5×10^6 cpm 32 P-labeled RNA from liver (+), 2×10^6 cpm from liver (-), 1×10^7 cpm from kidney (-), 1×10^7 cpm from spleen (-), and 1.6×10^6 cpm from spleen (+) to cDNA clones of albumin (Kioussis et al. 1981), tyrosine aminotransferase (Scherer et al. 1982), actin (Cleveland et al. 1980), and histone H4 (Seiler-Tuyns and Birnstein 1981) immobilized on nitrocellulose filters, and to a pBR322 derivative as a control. We also hybridized 0.01 of each of the above amounts of RNAs to a rRNA gene clone (Renkawitz et al. 1979). The filters were washed, and the RNase-resistant hybrids were autoradiographed for 3 days. RNAs from all transcription reactions hybridized to ribosomal DNA (rDNA), demonstrating that the action of α -amanitin was selective for RNA polymerase II. We used lower amounts of + RNAs in proportion to the relative levels of α -amanitin inhibition of total 32 P-labeled RNA synthesis in each reaction. We used one-fifth the level of liver - RNA relative to the levels of kidney and spleen - RNAs because previous experiments had shown that 10^7 cpm of liver - RNA would saturate the hybridization to albumin cDNA.

scription and found it to be about 1000-fold higher in liver than in kidney and, again, undetectable in spleen.

To determine whether the low kidney RNA signal represented specific transcription from the albumin promoter, we quantitated steady-state albumin mRNA levels by a primer extension assay. The extension products are displayed in Figure 2, along with a DNA sequence ladder of the albumin promoter region (Scott and Tilghman 1983). Each 10-fold serial dilution of total liver RNA gives rise to a major extension product that maps to a point 29 bp downstream of the albumin TATA sequence. Kidney RNA gives the same size extension product at 0.003 the level found in liver, demonstrating that the albumin promoter is transcriptionally active in kidney. We also observe similar low levels of the expected 2.2-kb albumin mRNA in kidney by Northern blot analysis (data not shown). Spleen RNA gives the expected extension product at 0.0005 the level found in liver, in addition to other extension products of larger and smaller sizes; no albumin mRNA is detected by Northern analysis (data not shown). We conclude that the albumin promoter is highly active in liver, slightly

Figure 2. Steady-state levels of mRNAs from the albumin promoter. Total RNAs were isolated from liver (L), kidney (K), and spleen (S), and the designated amounts were annealed to a 22-nucleotide end-labeled primer; the primer was extended with reverse transcriptase, and the extension products were electrophoresed on a denaturing 6% polyacrylamide gel. The liver RNA samples were mixed with 15 µg of *Escherichia coli* tRNA before annealing. The same primer was also used to generate a DNA sequence ladder (Sanger et al. 1977) from a cloned albumin gene template (Kioussis et al. 1981); thus, the displayed sequence is the complement of the mRNA. Albumin mRNA begins with the sequence ACC at the site shown by the arrowhead.

active in kidney, and rarely, if at all, specifically active in spleen.

Multiple tissue-specific hypersensitive sites encompass the albumin locus

We identified DNase I hypersensitive sites by isolating nuclei from different tissues of BALB/c mice and treating the nuclei with DNase I at relatively low levels of digestion. DNA was purified, fully cleaved with different restriction enzymes, fractionated by gel electrophoresis, transferred to nitrocellulose, and hybridized to either full length or indirect end-label (Wu 1980) DNA probes. The probes, mapping schemes, and autoradiogra-

phic results are shown in Figure 3. We also performed DNase I digestions of protein-free BALB/c mouse liver DNA to scan for hypersensitive sites due to enzyme affinity for particular DNA structures or sequences. We adjusted conditions of nuclease treatment to achieve similar extents of albumin gene digestion in each assay. We also show results of two different procedures for isolating liver nuclei to control for varying levels of endogenous nuclease activities.

As seen in Figure 3A, a strong liver-specific DNase I hypersensitive site occurs far upstream of the albumin gene. The enzyme appears to have a slight affinity for the same region of protein-free DNA. The indirect end-label probe in Figure 3B demonstrates that this site maps at -10.8 kb, relative to the albumin transcription start. Pinkert et al. (1987) also mapped a liver-specific hypersensitive site at -10.4 kb which, relative to standard markers on both of our gels, maps at the same position as the -10.8 -kb site we observe. They demonstrated further that sequences encompassing this site enhanced liver-specific transcription of a fusion gene introduced into transgenic mice.

We found other hypersensitive sites in liver upstream of the albumin transcription start, at -13.7 , -10 , and -3.5 kb, and at the promoter, -0.1 kb, which is especially hypersensitive (Fig. 3B and C). Kidney lacks all of the above hypersensitive sites except the one at the albumin promoter, which appears weakly hypersensitive. However, only kidney contains a site at -2.1 kb relative to the albumin promoter. None of the upstream hypersensitive sites is detectable in spleen, which apparently reflects the absence of albumin gene transcription in that tissue. Interestingly, protein-free DNA exhibits some hypersensitivity at the promoter (Fig. 3C, lane 2); this hypersensitivity is suppressed in the spleen (Fig. 3C, lanes 5–7). Although the albumin promoter is much more hypersensitive in liver than in kidney, the difference is not proportional to the much greater difference in albumin transcription rate ($1000\times$) between these tissues.

We also found hypersensitive sites within the transcribed region. At 6.6 kb downstream of the transcription start, cleavages occur both in liver and in kidney at the same intensity. Liver-specific sites also occur at positions 6.9, 8.7, 15, and 16.5 kb. The 16.5-kb site maps downstream of the final exon of albumin mRNA (Kioussis et al. 1981) but before the albumin gene terminator (J.-K. Liu and K.S. Zaret, unpubl.). Two sites, at 16 and 20.5 kb, occur in protein-free DNA and in all tissues tested; thus, these sites reflect nuclease affinity for a particular DNA structure or sequence that is dominant in chromatin. The 20.5-kb site is especially strong and occurs between the albumin gene and the α -fetoprotein gene, which maps another 13 kb downstream.

Simultaneous hypersensitivity at the albumin promoter and at the -3.5 -kb site

In the course of mapping hypersensitive sites by schemes that differ from those shown in Figure 3, we

discovered that our original approach suggested the -3.5 -kb site was only weakly hypersensitive in liver, compared with the promoter -0.1 -kb site (see Fig. 3C, lanes 16–18). As shown by a different indirect end-label

scheme in Figure 4, the -3.5 - and -0.1 -kb sites are actually of comparable hypersensitivity. The latter result is found when each site is probed separately and when each site occurs closest to the respective indirect end-

Figure 3. (See following page for legend.)

label probe (Fig. 4). Relative to the indirect end-label probe fragment *HindIII*–*EcoRI*, shown in map C of Figure 3, the –3.5-kb site is distal to the –0.1-kb site; thus, the –0.1-kb site precluded the ability to view fully all nuclease cleavages at the –3.5-kb site. This predicts the existence of a subband due to simultaneous nuclease cleavage, within an assay time point, at the –3.5- and –0.1-kb sites.

In previous hybridizations with the *HindIII*–*EcoRI* probe, we only detected subbands due to cleavage at either the –3.5- or –0.1-kb sites in liver chromatin (data not shown). In the hybridization of Figure 3C, we also included the *HindIII*–*NcoI* probe, as shown at the top of Figure 3. The additional probe clearly reveals the predicted subband due to simultaneous nuclease cleavage at both the –3.5- and –0.1-kb sites; this was observed only in liver chromatin. We were not able to detect simultaneous hypersensitivity at the albumin promoter and the –2.1-kb site in kidney. We scanned different autoradiographic exposures of the blot and quantitated signals of bands by normalizing to the specific activity and length of each probe that could hybridize. We used these data to compare subband signals to the parent restriction digest band from untreated nuclei (Fig. 3C, lane 12); the latter band reflects the total population of albumin genes studied. In the liver sample in Figure 3C, lane 18, the subband due to cleavage at only the –3.5-kb site represents ~2% of the total copies of albumin genes, whereas the subband due to cleavage at both the promoter and the –3.5-kb site represents 65% of the total. The values we find may be an underestimate because random nuclease cleavages between hypersensitive sites could reduce the ability to visualize a subband. We conclude that ~95% of the albumin genes that are detect-

ably hypersensitive at the –3.5-kb site can also be hypersensitive at the promoter, and over 60% of the albumin genes in liver nuclei can be hypersensitive at both of the sites simultaneously.

Time course of appearance of hypersensitivity in an assay

Next, we present mapping schemes that focus on the –0.1- and –3.5-kb hypersensitive sites. The autoradiographs in Figure 4, which display sequential hybridization to the same blot, indicate that the –0.1-kb site is cleaved by endogenous nuclease at the earliest time point in the assay (Fig. 4B, lane 2). In contrast, cleavages at the –3.5-kb site occur either at later time points or at early time points in the presence of added DNase I (Fig. 4A, lanes 4 and 5). (The samples in Fig. 4 are from a different chromatin preparation than the samples in Fig. 3). That is, the –3.5-kb site does not appear hypersensitive until the initial fraction of chromatin is cleaved. This predicts that simultaneous hypersensitivity at the albumin promoter and the –3.5-kb site should be detected only in digest points where both sites are cleaved strongly.

To test this prediction, samples from the DNase I assay shown in Figure 4 were run on an agarose gel without prior cleavage with restriction enzymes. The DNAs were transferred to nitrocellulose, and the same filter was hybridized sequentially to the three albumin gene probes A, B, and C, as shown at the top of Figure 5. Only probe B, which spans part of the region between the albumin promoter and the –3.5-kb site, hybridizes to the expected 3.4-kb-sized band in panel B. Furthermore, the band appears strongest in sample 4, which is

Figure 3. Tissue-specific DNase I hypersensitive sites. (Top) A map of the albumin gene showing only the restriction sites used to map positions of nuclease hypersensitivity in chromatin; the restriction enzymes used were *EcoRI* (E), *HindIII* (H), *HincII* (Hc), *KpnI* (K), *NcoI* (N), *PstI* (Ps), *PvuII* (Pv), *SacI* (Sa), and *StuI* (St). Black squares between the double lines represent exon sequences (Kioussis et al. 1981), and the wavy line indicates the transcribed region. Albumin transcription terminates in the dotted portion of the wavy line (J.-K. Liu and K.S. Zaret, unpubl.). The arrows and numbers designate distances, in kilobase pairs (kb), between DNase I hypersensitive sites and the albumin transcription start; letters above the arrows indicate the tissues in which the sites occur: (L) Liver; (K) kidney; (S) spleen; (D) protein-free DNA; (All) L + S + K + D. Sets of lines beneath the restriction map indicate the mapping strategies employed in panels A–F. Restriction sites at the endpoints of the labeled lines indicate the boundaries of the main restriction digest band displayed in the respective autoradiographs; thick lines represent the regions used as probes. Two probes were used simultaneously in panel C. Panels A, C, D, and F represent sequential hybridizations to the same blot. Arrows at the side of each panel indicate positions of subbands due to nuclease cleavage, and numbers adjacent to arrows indicate map positions of hypersensitive sites. As internal standards to accurately map positions of hypersensitive sites, we added marker lanes (designated M in each panel) of restriction digests of plasmid or genomic DNA. The marker bands, migrating below the main digest bands, are the results of cleavage at restriction sites corresponding to the endpoints of the thin lines beneath the main digest lines of the mapping strategies at the top. Each panel shows the results of restriction enzyme cleavage of 10- μ g DNA samples from nuclease assays, electrophoresis of the DNAs in agarose gels, transfer to nitrocellulose, hybridization to nick-translated DNA probes, and autoradiography for 2–7 days. Lanes 1, 2, and 3 display protein-free (purified) liver DNA digested with 0, 0.02, and 0.04 μ g/ml DNase I, respectively, at 23°C for 30 sec. Lanes 4–11 and 16–18 display DNA from nuclei prepared from the designated tissues by the method of Burch and Weintraub (1983), and lanes 12–15 display DNA from liver nuclei prepared by a different procedure (Kunnath and Locker 1985). Lanes 4 and 8 are from nuclear samples lysed without prewarming to 37°C. The absence of subbands in these samples indicates the absence of endogenous nuclease activity during the preparation of nuclei. We treated the nuclei at 37°C, as follows: (Lanes 5–7) with 0, 3.5, and 3.5 μ g/ml DNase I, respectively, for 4, 1, and 2.5 min; (lanes 9–11) with 0, 2, and 2 μ g/ml DNase I, respectively, for 3, 1, and 3 min; (lanes 12–15) with 0, 3, 5, and 8 μ g/ml DNase I, respectively, all for 30 sec; (lanes 16–18) with 0, 1.5, and 1.5 μ g/ml DNase I, respectively, for 3, 1, and 3 min. The nuclear incubations in the absence of added DNase I reveal the extent of endogenous nuclease cleavage within the given time period. Although some subbands appear in the absence of added DNase I, the enzyme caused a much faster appearance of all subbands shown (see lanes 12–13), except the subband due to cleavage at the 20.5-kb site. The samples in panel B, lane 9, and panel E, lanes 2, 3, and 9, were not digested to completion with restriction enzymes.

Figure 4. Time course of nuclease cleavages at the albumin promoter and the -3.5 -kb site. (Top) Symbols, restriction map, and probing strategies are similar to those in Figure 3; only sites used for mapping are shown. The autoradiographs display sequential hybridizations of two different probes to the same blot. Liver nuclei were prepared by the procedure of Burch and Weintraub (1983), and samples were treated as follows: (Lane 1) Nuclei were lysed immediately after homogenizing the cells; (lane 2) nuclei were washed twice as described (Burch and Weintraub 1983) and lysed without prewarming; (lanes 3 and 4) nuclei were warmed to 37°C and incubated for 20 sec and 3 min, respectively; (lanes 5–7) nuclei were warmed to 37°C and incubated further in the presence of DNase I at $1.5\text{ }\mu\text{g/ml}$ for 20 sec, 1 min, and 3 min, respectively. (Lanes 8–10) Protein-free liver DNA was digested for 30 sec at 23°C with DNase I at 0, 0.05, or $0.1\text{ }\mu\text{g/ml}$, respectively. DNA was purified and $10\text{-}\mu\text{g}$ DNA samples were digested with *Hind*III and analyzed on a 1.3% agarose gel, as in Figure 3. Multiple arrows indicate the complex cleavage pattern at each site.

the only digest point that contains strong cleavage at both the -3.5 - and -0.1 -kb sites.

Simultaneous hypersensitivity at the albumin promoter, enhancer, and other points in chromatin

All of the hypersensitive sites found in the chromatin samples in Figure 3 were also found in the samples used in Figure 5 by employing the indirect end-label assay (data not shown). Thus, we were confident of our ability to assign endpoints to the bands observed in the autoradiographs of Figure 5, as shown at the top. The indirect end-label assay demonstrated that the site at 6.6 kb downstream of the promoter, like the -3.5 -kb site, was cleaved at later time points in the assay; the other sites found in Figure 5 appeared strong at the earliest time points (data not shown). The temporal appearance of each double cleaved fragment in Figure 5 is consistent with these results, giving the appearance of bands that appear early (Fig. 5, lanes 2) or late (lanes 3 and 4), depending upon whether a cleavage is rate limiting.

The results demonstrate that individual albumin genes in liver are hypersensitive simultaneously at the promoter and at sites far downstream and upstream, including at the enhancer. Cleavages at sites -13.7 , -10.8 (the enhancer region), 15, and 20.5 kb occur simultaneously with the promoter at early digest time points (Fig. 5, lanes 2). At later digest points (Fig. 5, lanes 3 and 4), fragments cleaved at these sites appear to be chased to fragments containing cleavages at these sites and at the -3.5 - and 6.6-kb sites. The results do not simply reflect the stochastic nature of increased cleavage at closely spaced sites due to increased time of enzyme action, because we have shown that when the -3.5 - and 6.6-kb sites are mapped individually, they are cleaved later in the assay. Still, this does not prove that all of the widely separated sites are quantitatively

cleaved to give rise to the smaller digest fragments; the smaller digest fragments could be derived from separate populations of cells.

In Figure 5A and B, note that the band in lanes 2 and 3 due to hypersensitivity at both the albumin promoter and enhancer (the $-10.8/-0.1$ band) is chased to two bands of comparable intensity in lanes 3 and 4; in Figure 5A, to the $-10.8/-3.5$ band, and in Figure 5B, to the $-3.5/-0.1$ band. The data in Figure 3 show that the latter band can appear in nearly all gene copies that are cleaved at the -3.5 -kb site. Taken together, the data indicate that most of the genes that are hypersensitive at the -3.5 -kb site must also be hypersensitive simultaneously at the promoter and the enhancer. Thus, in liver, individual albumin genes possess an array of tissue-specific structures in chromatin that are present simultaneously, yet spaced over a 10-kb region.

Liver nuclear proteins bind specifically to DNA of the -3.5 -kb site

To study the role of the -3.5 -kb site, we tested the possibility that hypersensitivity could reflect binding of liver-specific nuclear proteins to the DNA. We prepared protein extracts (Dignam et al. 1983) from nuclei of liver, kidney, and spleen and used a gel retardation assay (Fried and Crothers 1981; Garner and Revzin 1981; Strauss and Varshavsky 1984) to detect factors that bind specifically to albumin gene segments. We first tested the integrity of our liver extracts and obtained specific binding to an albumin promoter fragment (data not shown). Next, we tested an end-labeled 380-bp *Stu*I–*Pvu*II fragment that fully spans the liver-specific hypersensitive site at -3.5 kb. As shown in Figure 6A, incubation with a liver nuclear extract causes the fragment to exhibit three complexes with retarded migration (la-

beled A, B, and C), in the presence of the nonspecific competitor, poly(dI/dC). The formation of all three complexes appears fully competed by a 30-fold molar excess of the same, unlabeled *StuI*–*PvuII* DNA, but competition is not observed with a 900-bp *Bam*HI fragment from within the albumin gene. We conclude that protein(s) in

liver nuclei can bind specifically to DNA of the –3.5-kb site.

We also tested kidney and spleen extracts for binding to the –3.5-kb site. As seen in Figure 6B, kidney nuclei contain about a 50-fold lower amount of factors that form complex C; these factors could be similar to or dif-

Figure 5. Nuclease-digested liver chromatin uncut by restriction enzymes. Liver chromatin samples were the same as those in Figure 4 but were not treated with restriction enzymes before electrophoresis in a 0.7% agarose gel at 1 V/cm. The DNA was transferred to nitrocellulose, and the same filter was hybridized sequentially to the three probes designated A, B, and C (top). The bands in the autoradiographs are due to simultaneous nuclease cleavage (within an assay time point) at two sites on individual copies of the albumin gene in chromatin. (Top) The positions of cleavages with respect to the albumin promoter are shown, with a representation of the bands that first appear early (lane 2) or later (lanes 3 and 4) in the assay shown below. The positions of cleavages could be determined with accuracy because we independently mapped all hypersensitive sites in these nuclear samples by the indirect end-labeling procedure (data not shown). A similar analysis of DNase I–cleaved protein-free DNA did not give rise to visible bands (data not shown).

Figure 6. Liver-abundant factors bind to -3.5 -kb site DNA in vitro. (A) Nuclear extracts from liver were incubated with an end-labeled, 380-bp fragment encompassing the -3.5 -kb hypersensitive site. Nonradioactive, specific (Spec.) and nonspecific (Nonsp.) competitors were added at the molar ratios shown. The binding reactions were separated by electrophoresis in a native 8% polyacrylamide gel and autoradiographed. Retarded migration of complexes A, B, and C is denoted at left. Complex C refers to two closely migrating bands. (B) Nuclear extracts from liver (L), kidney (K), and spleen (S) were treated as in panel A; (N and S) the addition of nonspecific and specific competitors, respectively. The relative levels of complex C formed in kidney and spleen extracts were the same whether or not a 30-fold excess nonspecific competitor was added (data not shown). (L + K and L + S) Mixed binding reactions with equal amounts of the two extracts.

ferent from the factors in the liver extracts. Spleen extracts form a complex migrating near position C at an abundance similar to liver. When liver extracts are mixed with extracts from either kidney or spleen, complexes A and B are observed, indicating that the absence of these complexes in the kidney and spleen reactions is not due to proteolysis. Thus, complex C could include factor(s) present at varying levels in different tissues, or different factors, whereas complexes A and B are liver specific. We conclude that liver-specific DNase I hypersensitivity at the -3.5 -kb site could reflect the specific binding of proteins in liver nuclei.

A heat-stable protein that binds the albumin promoter also binds the -3.5 -kb site in vitro

Specific protein-DNA contacts in complexes A, B, and C were revealed by partially methylating (Siebenlist and Gilbert 1980) purine residues of the *StuI*-*PvuII* fragment, performing the binding and gel retardation assay with liver nuclear proteins, and electroeluting the retarded complexes and free DNA (Hendrickson and Schleif 1985). As seen in Figure 7A, subsequent cleavage of the free DNA reveals a G > A sequence of the -3.5 -kb site. Similar cleavages of complexed DNAs show

footprints where methylation of three particular purine residues on the bottom strand interferes with protein binding. All three retarded complexes exhibit exactly the same footprint; the entire 380-bp fragment was scanned. The position of the footprint is in the promoter-proximal portion of the fragment, where the -3.5 -kb hypersensitive site occurs. When the methylation assay was repeated on more highly resolving sequencing gels (data not shown), the footprint sequence was determined unambiguously (Fig. 7B). Complexes A, B, and C each contain a protein, or a set of proteins, that binds to 14 bp containing a perfect inverted repeat of the sequence CAATCT.

The albumin promoter (-0.1 kb) has three separate binding sites for distinct proteins that have been reported to bind the sequence CAAT; all three sites appear important for albumin promoter activity. The most distal site, with respect to the transcription start, binds a protein related to nuclear factor 1 (NF1, Cereghini et al. 1987; Lichtsteiner et al. 1987). The next site, termed 'D' by Lichtsteiner et al. (1987), binds a heat-stable protein that is abundant in liver (Babiss et al. 1987; Cereghini et al. 1987) and appears similar or identical to a protein termed CAT binding protein (CBP, Graves et al. 1986) or enhancer binding protein (EBP, Johnson et al. 1987). The most proximal site contains the sequence CCAAT and

Figure 7. A heat-stable protein binds to both the -3.5 -kb site and the albumin promoter. (A) DMS-interference assay. DNA of the -3.5 -kb site was end labeled on the bottom strand, partially methylated with DMS, and used in a binding assay with liver nuclear proteins. Complexes A, B, and C and free DNA were electroeluted from the gel; the DNAs were cleaved at purines with NaOH ($G > A$), fractionated on a sequencing gel, and autoradiographed. Solid arrowheads indicate purines that prevent protein binding when methylated. (B) Sequence of the protected region. Arrows indicate a sequence that is rotationally symmetric about the axis of the vertical lines; triangles identify the bases in panel A. (C) Binding competition. Intact liver nuclear extracts (–), or extracts heated to 90°C before use (+), were used in a gel retardation assay with the labeled -3.5 -kb site DNA. Unlabeled competitor DNAs were -3.5 -kb site DNA (Spec); multimerized ($6\times$) oligonucleotide sequences comprising different binding sites of the albumin promoter (NF1, 'D', and CCAAT); DNA encompassing the albumin promoter from 14 to 315 bp upstream of the transcription start (promot.).

binds a cell-ubiquitous factor (Lichtsteiner et al. 1987; Raymondjean et al. 1988).

We heated our liver nuclear extract at 90°C for 10 min, pelleted and discarded the precipitated protein, and performed the gel retardation assay with -3.5 -kb site DNA. As seen in Figure 7C, all three retarded complexes could form in the heat-treated extract, and binding is competed by the same unlabeled fragment. We attribute the partial loss in binding activity to nonspecific association with the discarded protein precipitate. Heat-treated kidney and spleen extracts similarly form complex C (data not shown). A 301-bp fragment encompassing the albumin promoter competes for binding in a liver extract, although not as well as the specific competitor. We next performed competition reactions with multimerized ($6\times$) copies of double-stranded oligonucleotides that encompass albumin promoter-binding sites for each of the three factors described above. Figure 7C shows that only site D, and not the NF1 or CCAAT sites, competes for forming complexes A, B, and C in the liver extract. We conclude that similar liver-abundant factor(s) are present in each complex, and the factor(s) can bind in vitro to both the albumin promoter and the -3.5 -kb site; the same sequences are hypersensitive simultaneously in liver chromatin.

Discussion

We compared the transcription and chromatin structure of the albumin gene in three mouse tissues and found three distinct situations. In spleen, the gene is transcriptionally inactive, and the only hypersensitive sites evident are 16 and 20.5 kb downstream of the promoter. These sites are also hypersensitive in other tissues and in protein-free DNA, and therefore they must be due to DNA sequences or secondary structures that are dominant in chromatin. In kidney, the albumin promoter is active but at about 0.001 the level in liver. A kidney-specific hypersensitive site occurs 2.1 kb upstream; this site could be related to the low level of expression. In both liver and kidney, the gene is hypersensitive at the promoter and at a site 6.6 kb downstream, but hypersensitivity at the promoter is much greater in liver than in kidney. Only liver is hypersensitive simultaneously at the enhancer, promoter, and other distal sites in chromatin, indicating that factors causing hypersensitivity are present on each albumin gene at the same time.

As a model based on studies of the immunoglobulin enhancer (Klein et al. 1984, 1985; Wabl and Burrows 1984; Aguilera et al. 1985; Eckhardt and Birshstein 1985; Zaller and Eckhardt 1985; Atchison and Perry

1987), the albumin enhancer could set up a stable complex at the promoter during liver development and be dispensable thereafter. Our findings suggest that even if the enhancer is dispensable once it activates the promoter, the promoter-activation step may require an array of liver-specific elements to be present simultaneously in chromatin, and remnants of this array are preserved in the adult. We prefer a simpler model, where the enhancer, promoter, and possibly other elements work together actively in adult liver to effect albumin transcription.

The proportion of liver nuclei that is hypersensitive at the albumin promoter is about equal to the proportion of hepatocytes in the rodent liver (Greengard et al. 1972). Hypersensitivity at the promoter is likely to be caused by at least some of the various proteins that bind the promoter *in vitro* (Babiss et al. 1987; Cereghini et al. 1987; Lichtsteiner et al. 1987). The punctate hypersensitivity we observe over a 200-bp region of the promoter in chromatin (Fig. 4B) could reflect DNase I cleavages between bound proteins. Virtually all hepatocytes express albumin mRNA (Poliard et al. 1986); this, coupled with the hypersensitivity results, suggests that virtually all hepatocytes contain factors bound to the albumin promoter. Presumably, hypersensitivity at the albumin enhancer (Pinkert et al. 1987) also reflects factors bound to this liver-specific element.

In Pinkert's study, constructs that contained the enhancer juxtaposed to the promoter, with the -3.5 -kb site deleted, were expressed strongly in the liver of transgenic mice. However, a construct containing the region of the -3.5 -kb hypersensitive site, lacking the enhancer, directed expression that was low but detectably above levels of a construct with only 0.3 kb of upstream sequence. There was no evidence that the -3.5 -kb region could function as an enhancer. Previous studies found a liver-specific hypersensitive site 2.8 kb upstream of the rat albumin promoter (Babiss et al. 1986; Turcotte et al. 1986; Nahon et al. 1987; Tratner et al. 1987); this site is in a position similar to the site found at -3.5 kb in mice by Pinkert et al. (1987) and in this study. Rodent hepatoma cell lines transcribe the albumin gene at a much lower rate than liver (Clayton et al. 1985b); in such cells the gene is weakly hypersensitive at the promoter, and not at all at the expected -2.8 -kb (Babiss et al. 1986; Nahon et al. 1987) or -3.5 -kb (Y. Bergman and K. Zaret, unpubl.) sites. Thus, hypersensitivity at the -3.5 -kb site is correlated with high albumin gene transcription rates, but the region does not appear to function as a typical enhancer element.

Liver nuclear proteins form three distinct complexes with DNA of the -3.5 -kb site. Complexes A and B are liver specific, whereas complex C forms at low levels in kidney extracts and at levels comparable to liver in spleen extracts. The heat resistance of factor(s) causing complex C in all three extracts suggests that the factor(s) could be related. The similar dimethylsulfate (DMS) interference, heat resistance, and competition in forming complexes A, B, and C in liver extracts strongly implicate related binding activities. Surprisingly, binding

specificity for the -3.5 -kb site is similar to that of a liver-abundant factor that binds site D of the albumin promoter (Babiss et al. 1987; Cereghini et al. 1987; Lichtsteiner et al. 1987); site D is required for full promoter activity (Heard et al. 1987; Lichtsteiner et al. 1987).

The relative levels of complex C formed in liver and kidney extracts are consistent with reported levels of the D site factor (Babiss et al. 1987; Cereghini et al. 1987). The sum of complexes A, B, and C formed in liver is greater than complex C in spleen; similar relative levels of the D site factor in those tissues are seen in the data of Cereghini et al. (1987). Using a different extract preparation than that of Cereghini and ourselves, Lichtsteiner et al. (1987) find the D site factor at a much lower level in spleen. Based on its liver abundance, heat resistance, and binding specificities, the D site factor was suggested by Lichtsteiner et al. (1987) to be identical to the CBP/EBP protein (Graves et al. 1986; Johnson et al. 1987). Strikingly, the heat-stable factor(s) that bind the -3.5 -kb site recognize a rotationally symmetric repeat of the sequence CAATCT; Graves et al. (1986) have argued that CBP/EBP-binding sites possess such rotational symmetry. Although we do not know whether the -3.5 -kb binding protein is identical to CBP/EBP, it is clear that a protein with similar characteristics can bind to both the albumin promoter and the -3.5 -kb site.

The different sizes of liver complexes A, B, and C could be due to multimeric forms or proteolytic products of the same protein; proteolyzed forms of CBP/EBP retain similar DNA-binding specificities (Johnson et al. 1987). Another possibility is that complexes A and B include liver-specific proteins that bind the same sequence as a cell-ubiquitous protein that forms complex C. Distinct cell-specific and ubiquitous proteins with identical octamer DNA-binding specificities have been described (Landolfi et al. 1986; Staudt et al. 1986; Fletcher et al. 1987; Scheidereit et al. 1987). Alternatively, other heat-stable, liver-specific factors could bind to the factor that binds CAATCT, or bind to the DNA probe but not be affected by DMS methylation of the bottom strand. Indeed, the extended DNase I cleavage pattern we observe at the -3.5 -kb site in liver nuclei (Fig. 4A) could be due to the binding of multiple factors. Factor(s) unique to complexes A and B could cause the -3.5 -kb site to be hypersensitive only in liver, and the factor(s) may function differently than that in complex C.

CBP/EBP, like the lymphoid-specific octamer-binding protein (Singh et al. 1986; Sen and Baltimore 1986), binds to both promoter and enhancer sequences. We describe a protein with characteristics of CBP/EBP that can bind to the promoter and an upstream site whose hypersensitivity is associated with a high level of albumin gene transcription. If similar proteins bound simultaneously to these sites *in vivo*, association of the proteins could facilitate looping together distal regions of DNA (Dunn et al. 1984; Ptashne 1986). The function of such a structure could be to mediate the action of the highly distal enhancer or to create a local chromatin configura-

tion compatible with a high transcription rate in hepatocyte cells. We are currently investigating these possibilities.

Materials and methods

Transcription rate analysis

Nuclei were prepared from liver, kidney, and spleen of a BALB/c mouse (Charles River Labs), and run-on assays and hybridizations were performed exactly as described by Clayton et al. (1985a), except that we included complementary ³H-labeled RNA of albumin cDNA in the hybridizations to monitor reaction efficiency. The cRNA was synthesized essentially as described by McKnight and Palmiter (1979), and it revealed our hybridization efficiency to be ~20%. After hybridization, the washed and RNase-treated filters were exposed to Kodak XAR film with Dupont Cronex intensifying screens for 1–5 days. Signal intensities of different exposures were measured with an LKB Ultrascan laser densitometer. Transcription rates were quantitated by subtracting the background hybridization to pBR3 and normalizing to the level of rRNA gene transcription.

Steady-state RNA analysis

Total RNAs were isolated from BALB/c mouse tissues by the guanidinium/cesium chloride method (Glisin et al. 1974; Chirgwin et al. 1979). Primer extension was performed with an end-labeled oligonucleotide (McKnight and Kingsbury 1982). The oligonucleotide was made with a Biosearch 8600 synthesizer; its sequence was 5'-GCTTCTCGGCGAAACA-CACCCC-3'. The DNA sequence ladder in Figure 2 was generated by the dideoxy method (Sanger et al. 1977) with an end-labeled primer.

Isolation of nuclei and DNase I treatment

Nuclei for chromatin studies were prepared by either (1) the procedure of Burch and Weintraub (1983), which gave the strongest subbands but higher levels of endogenous nucleases, or (2) a modification of the procedure of Kunath and Locker (1985), which gave weaker subbands but lower levels of endogenous nucleases. The latter procedure was modified as follows: Fresh BALB/c mouse livers were minced in an ice-cold solution of SSC + 10 mM Tris [0.15 M NaCl, 0.015 M citrate, 10 mM Tris (pH 7.4)], as described by Burch and Weintraub (1983) and then homogenized in a solution of 60 mM KCl, 15 mM Tris (pH 7.4), 0.5 mM dithiothreitol, 0.1 mM phenylmethylsulfonylfluoride, and 0.5 M sucrose with a teflon pestle in a Dounce homogenizer. The nuclear suspensions were layered on step gradients composed of 0.5 M, 1.5 M, and 1.7 M sucrose in homogenization buffer and pelleted by centrifugation of 11,000 rpm for 1 hr.

Nuclei prepared by either method were suspended in a solution of 10 mM Tris (pH 7.4), 10 mM NaCl, and 3 mM MgCl₂ in a glass tube on ice. Nuclear concentrations were adjusted to OD₂₆₀ units, as assayed by dilution into 1% SDS. Nuclei were warmed to 37°C while shaking gently for 45 sec, adjusted to 0.1 mM CaCl₂, and treated further as described in the legends to Figures 3 and 4. DNase I was from Cooper Biomedical. Digestion was terminated by adding an equal volume of a solution of 20 mM Tris (pH 7.4), 200 mM NaCl, 2 mM EDTA, 1% SDS, and 200 µg/ml proteinase K. After overnight incubation at 37°C, one volume of a 10 mM Tris (pH 7.4), 1 mM EDTA (TE) solution was added and the DNA was extracted once with phenol and three times with chloroform and precipitated with Na acetate and ethanol. DNA precipitates were spooled out with a glass micropipette and resuspended in TE.

Hybridization analysis of DNA

Restriction digests, electrophoresis, transfer of DNA to nitrocellulose filters, and hybridization to nick-translated probes were as described previously (Zaret and Yamamoto 1984). All probes were DNA restriction fragments of the cloned mouse albumin locus (Kioussis et al. 1981) purified from agarose gels. Specific activities were 0.5×10^9 to 2×10^9 cpm/µg DNA. Filters were stripped of probe by wetting them, pouring boiling water on them, and gently shaking them for 20 min; after this treatment, they were ready for prehybridization and hybridization as before. Filters were exposed to film with screens at –80°C for 2–7 days. Autoradiographs were scanned by laser densitometry.

Preparation of nuclear extracts

Nuclei were isolated from tissues combined from six BALB/c mice, as described by Fritton et al. (1980); extracts were prepared according to Dignam et al. (1983). Nuclear proteins were concentrated at 4°C by adding (NH₄)₂SO₄ slowly to 0.3 g/ml, stirring for 1 hr, and pelleting the precipitate at 10,000 rpm in an SS34 rotor for 20 min. Pellets were resuspended in 1 ml of buffer D (Dignam et al. 1983) for liver and 200 µl for kidney and spleen. Suspensions were dialyzed overnight at 4°C against two changes of buffer D, spun in a microfuge to remove aggregates, and frozen in liquid N₂ and stored at –85°C. Extracts were heat treated by incubating at 90°C for 10 min, cooling on ice for 5 min, pelleting the precipitate in a microfuge for 5 min, and transferring the supernatant to a fresh tube.

Gel retardation analysis

All binding reactions were performed in a 20-µl solution of 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, and 1% Ficoll, containing 3–9 µg poly(dI/dC) (Pharmacia), 3 µg of nuclear extract, and competitor DNAs as indicated in Figures 6 and 7. The mixtures were incubated at room temperature for 10 min, 0.4-ng (15,000 cpm) amounts of probe were added, and incubations proceeded for an additional 20 min. Binding reactions were loaded onto native 8% polyacrylamide gels [acrylamide : bisacrylamide of 30 : 1] that were prerun in TBE buffer (89 mM Tris base, 74 mM boric acid, 0.88 mM EDTA) at 11 V/cm for 2 hr and then run at the same voltage, all without buffer circulation. Gels were dried and autoradiographed with a screen at –80°C for 12–24 hr. The probe in Figures 6 and 7 was a 380-bp *StuI*–*PvuII* fragment of albumin locus DNA (Kioussis et al. 1981) that was cloned into the *SmaI* site of pUC19, retrieved by digestion with *EcoRI* and *XbaI*, and end-labeled with [α-³²P]dATP, according to Maniatis et al. (1982). Different nuclear preparations and electrophoresis conditions gave similar results, whereas labeled DNA fragments of pUC plasmid exhibited no specific binding (data not shown).

Competitor DNAs in Figure 7C were as follows: The albumin promoter fragment extends from 14 to 315 bp upstream of the transcription start site. The NF1, D, and CCAAT site DNAs were made from complementary synthetic oligonucleotides containing the albumin promoter sequences ACAACTTTTGGCAAAGAT, ATGATTTTGTAAATGGGGTAG, and GGGGTAGGAACCAATGAAA, respectively, based upon the footprinting data of Babiss et al. (1987). Three bases were added to the 5' end of each strand during synthesis to permit head-to-tail annealing. The oligos were annealed, ligated, filled in at the ends with Klenow polymerase, and size fractionated on a native polyacrylamide gel. Multimers of six were eluted from the gel, quantitated, and used as competitor DNAs in binding reactions.

DMS footprinting

End-labeled DNA was partially methylated with DMS, as described by Rosales et al. (1987), and used in a binding reaction that was scaled up fivefold. After electrophoresis and autoradiography of the wet gel, bands were excised and electroeluted, and the DNA was cleaved at modified purines ($G > A$), following the protocol of Hendrickson and Schleif (1985). The cleavage products were separated on denaturing 6% polyacrylamide gels and exposed to X-ray film with screens.

Acknowledgments

We are grateful to Dr. Shirley Tilghman for providing cloned mouse albumin DNA used in this study. Dr. John Thompson provided advice on the gel shift assay, and Bettina Franz helped with the oligonucleotide syntheses. The rDNA and histone H4 clones were from Drs. Susan Gerbi and Rudi Grosschedl, respectively. We thank Kimberly Stevens for technical assistance, Michael DiPersio, David Jackson, and Anne Kerrebrock for comments on the manuscript, and Marybeth Tremblay for help in preparing the manuscript. This work was supported by a grant to K.Z. from the National Institutes of Health (GM36477). K.Z. is a recipient of a Searle Scholars Award.

References

- Aguilera, R.J., T.J. Hope, and H. Sakano. 1985. Characterization of immunoglobulin enhancer deletions in murine plasmacytomas. *EMBO J.* **4**: 3689–3693.
- Atchison, M.L. and R.P. Perry. 1987. The role of the kappa enhancer and its binding factor NF-kappa B in the developmental regulation of kappa gene transcription. *Cell* **48**: 121–128.
- Babiss, L.E., R.S. Herbst, A.L. Bennett, and J.E. Darnell, Jr. 1987. Factors that interact with the rat albumin promoter are present both in hepatocytes and other cell types. *Genes Dev.* **1**: 256–267.
- Babiss, L.E., A. Bennett, J.M. Friedman, and J.E. Darnell, Jr. 1986. DNase I-hypersensitive sites in the 5'-flanking region of the rat serum albumin gene: Correlation between chromatin structure and transcriptional activity. *Proc. Natl. Acad. Sci.* **83**: 6504–6508.
- Benezra, R., C.R. Cantor, and R. Axel. 1986. Nucleosomes are phased along the mouse β -major globin gene in erythroid and nonerythroid cells. *Cell* **44**: 697–704.
- Burch, J.B.E. and H. Weintraub. 1983. Temporal order of chromatin structural changes associated with activation of the major chicken vitellogenin gene. *Cell* **33**: 65–76.
- Cereghini, S., M. Raymondjean, A.G. Carranca, P. Herbolme, and M. Yaniv. 1987. Factors involved in control of tissue-specific expression of albumin gene. *Cell* **50**: 627–638.
- Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**: 5294–5299.
- Clayton, D.F., A.L. Harrelson, and J.E. Darnell, Jr. 1985a. Dependence of liver-specific transcription on tissue organization. *Mol. Cell. Biol.* **5**: 2623–2632.
- Clayton, D.F., M. Weiss, and J.E. Darnell, Jr. 1985b. Liver-specific RNA metabolism in hepatoma cells: Variations in transcription rates and mRNA levels. *Mol. Cell. Biol.* **5**: 2633–2641.
- Cleveland, D.W., M.A. Lopata, R.J. MacDonald, N.J. Cowan, W.J. Rutter, and M.W. Kirschner. 1980. Number and evolutionary conservation of α - and β -tubulin and cytoplasmic β - and γ -actin genes using specific cloned cDNA probes. *Cell* **20**: 95–105.
- Dignam, J.D., R.M. Lebowitz, and R.G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11**: 1475–1489.
- Dunn, T.M., S. Hahn, S. Ogden, and R.F. Schleif. 1984. An operator at –280 base pairs that is required for repression of *arab* operon promoter: Addition of DNA helical turns between the operator and promoter cyclically hinders repression. *Proc. Natl. Acad. Sci.* **81**: 5017–5020.
- Eckhardt, L.A. and B.K. Birshstein. 1985. Independent immunoglobulin class-switch events occurring in a single myeloma cell line. *Mol. Cell. Biol.* **5**: 856–868.
- Eissenberg, J.C., I.L. Cartwright, G.H. Thomas, and S.C.R. Elgin. 1985. Selected topics in chromatin structure. *Annu. Rev. Genet.* **19**: 485–536.
- Emerson, B.M. and G. Felsenfeld. 1984. Specific factor conferring nuclease hypersensitivity at the 5' end of the chicken adult β -globin gene. *Proc. Natl. Acad. Sci.* **81**: 95–99.
- Fletcher, C., N. Heintz, and R.G. Roeder. 1987. Purification and characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone H2b gene. *Cell* **51**: 773–781.
- Fried, M.G. and D.M. Crothers. 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. *Nucleic Acids Res.* **9**: 6505–6525.
- Fritton, H.P., A.E. Sippel, and T. Igo-Kemenes. 1980. Nuclease-hypersensitive sites in the chromatin domain of the chicken lysozyme gene. *Nucleic Acids Res.* **11**: 3467–3485.
- Fritton, H.P., T. Igo-Kemenes, J. Nowock, U. Strech-Jurk, M. Theisen, and A.E. Sippel. 1984. Alternative sets of DNase I-hypersensitive sites characterize the various functional states of the chicken lysozyme gene. *Nature* **311**: 163–165.
- Garner, M.M. and A. Revzin. 1981. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions. *Nucleic Acids Res.* **9**: 3047–3060.
- Glisin, V., R. Cryenjakov, and C. Byus. 1974. Ribonucleic acid isolated by cesium chloride centrifugation. *Biochemistry* **3**: 2633–2637.
- Graves, B.J., P.F. Johnson, and S.L. McKnight. 1986. Homologous recognition of a promoter domain common to the MSV LTR and the HSV tk gene. *Cell* **44**: 565–576.
- Greengard, O., M. Federman, and W.E. Knox. 1972. Cytophotometry of developing rat liver and its application to enzymic differentiation. *J. Cell Biol.* **52**: 261–272.
- Heard, J.-M., P. Herbolme, M.-O. Ott, A. Mottura-Rollier, M. Weiss, and M. Yaniv. 1987. Determinants of rat albumin promoter tissue specificity analyzed by an improved transient expression system. *Mol. Cell Biol.* **7**: 2425–2434.
- Hendrickson, W. and R. Schleif. 1985. A dimer of AraC protein contacts three adjacent major groove regions of the *araI* DNA site. *Proc. Natl. Acad. Sci.* **82**: 3129–3133.
- Jackson, P.D. and G. Felsenfeld. 1985. A method for mapping intranuclear protein-DNA interactions and its application to a nuclease hypersensitive site. *Proc. Natl. Acad. Sci.* **82**: 2296–2300.
- Johnson, P.F., W.H. Landschulz, B.J. Graves, and S.L. McKnight. 1987. Identification of a rat liver nuclear protein that binds to the enhancer core element of three animal viruses. *Genes Dev.* **1**: 133–146.
- Jongstra, J., T.L. Reudelhuber, P. Oudet, C. Benoist, C.-B. Chae, J.-M. Jeltsch, D.J. Mathis, and P. Chambon. 1984. Induction of altered chromatin structures by simian virus 40 enhancer and promoter elements. *Nature* **307**: 708–714.

- Kioussis, D., F. Eiferman, P. van de Rijn, M.B. Gorin, R.S. Ingram, and S.M. Tilghman. 1981. The evolution of the α -fetoprotein and albumin. II. The structures of the α -fetoprotein and albumin genes in the mouse. *J. Biol. Chem.* **256**: 1960–1967.
- Klein, S., F. Sablitzky, and A. Radbruch. 1984. Deletion of the IgH enhancer does not reduce immunoglobulin heavy chain production of a hybridoma IgD class switch variant. *EMBO J.* **3**: 2473–2476.
- Klein, S., T. Gerster, D. Picard, A. Radbruch, and W. Schaffner. 1985. Evidence for transient requirement of the IgH enhancer. *Nucleic Acids Res.* **13**: 8901–8912.
- Kunnath, L. and J. Locker. 1985. DNase I sensitivity of the rat albumin and α -fetoprotein genes. *Nucleic Acids Res.* **13**: 115–129.
- Landolfi, N.F., J.D. Capra, and P.W. Tucker. 1986. Interaction of cell-type-specific nuclear proteins with immunoglobulin V_H promoter region sequences. *Nature* **323**: 548–553.
- Larsen, A. and H. Weintraub. 1982. An altered DNA conformation detected by S1 nuclease occurs at specific regions in active chick globin chromatin. *Cell* **29**: 609–622.
- Lichtsteiner, S., J. Wuarin, and U. Schibler. 1987. The interplay of DNA-binding proteins on the promoter of the mouse albumin gene. *Cell* **51**: 963–973.
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- McGhee, J.D., W.I. Wood, M. Dolan, J.D. Engel, and G. Felsenfeld. 1981. A 200 base pair region at the 5' end of the chicken adult β -globin gene is accessible to nuclease digestion. *Cell* **27**: 45–55.
- McKnight, S.L. and R. Kingsbury. 1982. Transcriptional control signals of a eukaryotic protein-coding gene. *Science* **217**: 316–324.
- McKnight, G.S. and R.D. Palmiter. 1979. Transcriptional regulation of the ovalbumin and conalbumin genes by steroid hormones in chick oviduct. *J. Biol. Chem.* **254**: 9050–9058.
- McKnight, G.S., D.C. Lee, and R.D. Palmiter. 1980. Transferrin gene expression: Regulation of mRNA transcription in chick liver by steroid hormones and iron deficiency. *J. Biol. Chem.* **255**: 148–153.
- Nahon, J.-L., A. Venetianer, and J.M. Sala-Trepat. 1987. Specific sets of DNase I-hypersensitive sites are associated with the potential and overt expression of the rat albumin and α -fetoprotein genes. *Proc. Natl. Acad. Sci.* **84**: 2135–2139.
- Nickol, J.M. and G. Felsenfeld. 1983. DNA conformation at the 5' end of the chicken adult β -globin gene. *Cell* **35**: 467–477.
- Pachnis, V., A. Belayew, and S.M. Tilghman. 1984. Locus unlinked to α -fetoprotein under the control of the murine *raf* and *Rif* genes. *Proc. Natl. Acad. Sci.* **81**: 5523–5527.
- Parslow, T.G. and D.K. Granner. 1982. Chromatin changes accompany immunoglobulin kappa gene activation: A potential control region within the gene. *Nature* **299**: 449–451.
- Pinkert, C.A., D.M. Ornitz, R.L. Brinster, and R.D. Palmiter. 1987. An albumin enhancer located 10 kb upstream functions along with its promoter to direct efficient, liver-specific expression in transgenic mice. *Genes Dev.* **1**: 268–276.
- Poliard, A.M., D. Bernuau, I. Tournier, L.G. Legres, D. Schoevaert, G. Feldmann, and J.M. Sala-Trepat. 1986. Cellular analysis by in situ hybridization and immunoperoxidase of alpha-fetoprotein and albumin gene expression in rat liver during the perinatal period. *J. Cell Biol.* **103**: 777–786.
- Powell, D.J., J.M. Friedman, A.J. Ouletter, K.S. Krauter, and J.E. Darnell, Jr. 1984. Transcriptional and post-transcriptional control of specific messenger RNAs in adult and embryonic liver. *J. Mol. Biol.* **179**: 21–35.
- Ptashne, M. 1986. Gene regulation by proteins acting nearby and at a distance. *Nature* **322**: 697–701.
- Raymondjean, M., S. Cereghini, and M. Yaniv. 1988. Several distinct 'CCAAT' box binding proteins coexist in eukaryotic cells. *Proc. Natl. Acad. Sci.* **85**: 757–761.
- Renkawitz, R., S.A. Gerbi, and K.H. Glatzer. 1979. Ribosomal DNA of the fly *Sciara coprophila* has a very small and homogeneous repeat unit. *Mol. Gen. Genet.* **173**: 1–13.
- Rosales, R., M. Vigneron, M. Macchi, I. Davidson, J.H. Xiao, and P. Chambon. 1987. *In vitro* binding of cell-specific and ubiquitous nuclear proteins to the octamer motif of the SV40 enhancer and related motifs present in other promoters and enhancers. *EMBO J.* **6**: 3015–3025.
- Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
- Scheidereit, C., A. Heguy, and R.G. Roeder. 1987. Identification and purification of a human lymphoid-specific octamer-binding protein (OTF-2) that activates transcription of an immunoglobulin promoter *in vitro*. *Cell* **51**: 783–793.
- Scherer, G., W. Schmid, C.M. Strange, W. Rowekamp, and G. Schutz. 1982. Isolation of cDNA clones coding for rat tyrosine aminotransferase. *Proc. Natl. Acad. Sci.* **79**: 7205–7208.
- Schon, E., T. Evans, J. Welsh, and A. Efstratiadis. 1983. Conformation of promoter DNA: Fine mapping of S1-hypersensitive sites. *Cell* **45**: 837–848.
- Scott, R.W. and S.M. Tilghman. 1983. Transient expression of a mouse α -fetoprotein minigene: Deletion analyses of promoter function. *Mol. Cell. Biol.* **3**: 1295–1309.
- Seibenlist, U.L. and W. Gilbert. 1980. Contact between *Escherichia coli* RNA polymerase and an early promoter of phage T7. *Proc. Natl. Acad. Sci.* **77**: 122–126.
- Seiler-Tuyns, A. and M.L. Birnstein. 1981. Structure and expression in L-cells of a cloned H4 histone gene of the mouse. *J. Mol. Biol.* **151**: 607–625.
- Sen, R. and D. Baltimore. 1986. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* **46**: 705–716.
- Shermoen, A.W. and S.K. Beckendorf. 1982. A complex of interacting DNase I-hypersensitive sites near the *Drosophila* glue protein gene, *Sgs4*. *Cell* **29**: 601–607.
- Singh, H., R. Sen, D. Baltimore, and P.A. Sharp. 1986. A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes. *Nature* **319**: 154–158.
- Stalder, J., A. Larsen, J.D. Engel, M. Dolan, M. Groudine, and H. Weintraub. 1980. Tissue-specific DNA cleavages in the globin chromatin domain introduced by DNase I. *Cell* **20**: 451–460.
- Staudt, L.M., H. Singh, R. Sen, T. Wirth, P.A. Sharp, and D. Baltimore. 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. *Nature* **323**: 640–644.
- Strauss, F. and A. Varshavsky. 1984. A protein binds to a satellite DNA repeat at three specific sites that would be brought into mutual proximity by DNA folding in the nucleosome. *Cell* **37**: 889–901.
- Tilghman, S.M. and A. Belayew. 1982. Transcriptional control of the murine albumin/ α -fetoprotein locus during development. *Proc. Natl. Acad. Sci.* **79**: 5254–5257.
- Tratner, I., J.-L. Nahon, J.M. Sala-Trepat, and A. Venetianer. 1987. Albumin and α -fetoprotein gene transcription in rat hepatoma cell lines is correlated with specific DNA hypomethylation and altered chromatin structure in the 5' region. *Mol. Cell. Biol.* **7**: 1856–1864.

- Turcotte, B., M. Guertin, M. Chevette, H. LaRue, and L. Belanger. 1986. DNase I hypersensitivity and methylation of the 5'-flanking region of the α -fetoprotein gene during developmental and glucocorticoid-induced repression of its activity in rat liver. *Nucleic Acids Res.* **14**: 9827–9841.
- Wabl, M.R. and P.D. Burrows. 1984. Expression of immunoglobulin heavy chain at a high level in the absence of a proposed immunoglobulin enhancer element in *cis*. *Proc. Natl. Acad. Sci.* **81**: 2452–2455.
- Wu, C. 1980. The 5' ends of *Drosophila* heat shock genes in chromatin are hypersensitive to DNase I. *Nature* **286**: 854–860.
- Zaller, D.M. and L.A. Eckhardt. 1985. Deletion of a B-cell-specific enhancer affects transfected, but not endogenous, immunoglobulin heavy-chain gene expression. *Proc. Natl. Acad. Sci.* **82**: 5088–5092.
- Zaret, K.S. and K.R. Yamamoto. 1984. Reversible and persistent changes in chromatin structure accompany activation of a glucocorticoid-dependent enhancer element. *Cell* **38**: 29–38.

The mouse albumin promoter and a distal upstream site are simultaneously DNase I hypersensitive in liver chromatin and bind similar liver-abundant factors in vitro.

J K Liu, Y Bergman and K S Zaret

Genes Dev. 1988, **2**:

Access the most recent version at doi:[10.1101/gad.2.5.528](https://doi.org/10.1101/gad.2.5.528)

References

This article cites 71 articles, 28 of which can be accessed free at:
<http://genesdev.cshlp.org/content/2/5/528.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).