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DNaseI HYPERSENSITIVE SITES AT THE 3′ END OF THE HUMAN APOLIPOPROTEIN B GENE

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The chromatin structure of the 3′ end of the human apolipoprotein B gene has been examined. Two DNaseI hypersensitive sites were present in nuclei from liver-derived HepG2 cells and intestine-derived CaCo-2 cells, in which the apo-B gene is transcriptionally active, but were absent from HeLa cells, where the gene is not expressed, and from free DNA. The first DNaseI hypersensitive site was localized to an A/T-rich hypervariable region in a segment enriched in recognition sites for topoisomerase II and known to participate in anchoring the 3′ end of the gene to the nuclear matrix. The second DNaseI hypersensitive site resided in the 3′ untranslated portion of the gene. Furthermore, nucleosomes were present along a 1.4-kilobase (*Hin*dIII-*Bam*HI) segment of DNA containing the two 3′ DNaseI hypersensitive sites, and a static array of nucleosomes was present along the A/T-rich hypervariable region.
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In eukaryotic cells, gene expression is regulated by a complex set of interactions between cis-acting DNA sequences and trans-acting protein factors. In chromatin, these protein-DNA interactions can generate DNaseI hypersensitive (DH) sites and sometimes occur near chromosomal loops (for a review, see Ref. 1). DNaseI hypersensitive sites are generally found at the 5′ ends of actively expressed genes, although some genes also possess DH sites at their 3′ ends (2). The 5′ DH sites have the potential to form an altered DNA secondary structure, as evidenced by their sensitivity to single-stranded nucleases (3). These altered DNA structures may be recognized *in vivo* by specific factors involved in the initiation of transcription.

The mechanisms involved in the termination of transcription in animal cells remain obscure. Data obtained from studies of several genes (4) show that RNA polymerase II reads through polyadenylation signals, terminating the primary transcript at some distance beyond

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Abbreviations: DH, DNaseI hypersensitive; apo-, apolipoprotein; HVR, hypervariable region; bp, base pair(s).

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the last codon in the mRNA. Very little is known about the specific chromatin organization of the region containing the sequences involved in the termination of transcription. For instance, DH sites located 3′ of the ovalbumin and lysozyme genes have been found in chromatin of laying hen oviduct, but these DH sites have not been correlated with specific DNA sequences or gene activity (5, 6). On the other hand, Bellard *et al.* (7) have shown that the ovalbumin gene in oviduct chromatin displays a hypersensitive region at the 3′ end that is hormone-dependent and that correlates with the transcriptional activity of the gene. Furthermore, the 3′ moiety of the ovalbumin gene and its flanking regions show an altered nucleosomal pattern in actively expressing cells as compared with inactive cells (7). The presence of an altered chromatin structure in the region of the ovalbumin gene containing the polyadenylation and transcription termination sequences raises the question as to whether this is a general feature of genes coding for mRNA, reflecting the binding of proteins involved in these processes.

Our interest lies in the mechanisms responsible for the control of expression of the human apolipoprotein (apo-) B gene, which is expressed only in hepatic and intestinal cells and codes for a protein with a fundamental role in lipid metabolism and transport. The apo-B gene resides in a 47.5-kilobase DNaseI sensitive chromatin domain in the nuclei of liver hepatoma (HepG2) and colon carcinoma (CaCo-2) cells (8). It contains 29 exons and 28 introns (9). We have recently localized DNA sequences within and near the apo-B gene that are involved in the interaction with the nuclear matrix (8). That study revealed that the apo-B gene is anchored to the nuclear matrix at both its 5′ and 3′ ends. At each end, A/T-rich sequences resembling the recognition site for topoisomerase II are involved in the anchoring process. The 3′ matrix attachment region was localized to an A/T-rich hypervariable region (HVR) composed of 25-52 copies of two different 15-base pair (bp) repeats (10). Because this HVR represents the boundary of the apo-B gene where transcription ends, we sought to determine whether any DH sites were present at the 3′ end of the gene and whether a normal or altered nucleosomal array was evident.

MATERIALS AND METHODS

Tissue culture, isolation of nuclei and nuclease digestion, gel electrophoresis, and Southern blotting

These procedures were performed as described in Levy-Wilson *et al.* (11).

DNA probes

The 3′ DNA fragments used as probes were isolated from apo-B genomic subclones by digestion with restriction enzymes followed by purification of the DNA fragments on

polyacrylamide gels. Labeling was by random priming using a kit from Bethesda Research Laboratories (Bethesda, Maryland).

RESULTS AND DISCUSSION

DNase I hypersensitivity in the 3′ end of the human apolipoprotein B gene

To determine whether any DH sites were present in the 3′ end of the human apo-B gene in the vicinity of the A/T-rich HVR, nuclei from HepG2, CaCo-2, and HeLa cells were digested with increasing amounts of DNaseI. DNA was then purified and digested with *Eco*RI and *Stu*I. Digestion products were fractionated on 1.2% agarose gels and transferred to nitrocellulose filters (11). The blots were hybridized to an end-labeled probe comprising unique sequences from exon 29 of the apo-B gene.

The results illustrated in Figure 1 show that in HepG2 cells, limited DNaseI digestion of nuclei (leftmost panel), but not of free DNA (rightmost panel), generated two bands smaller

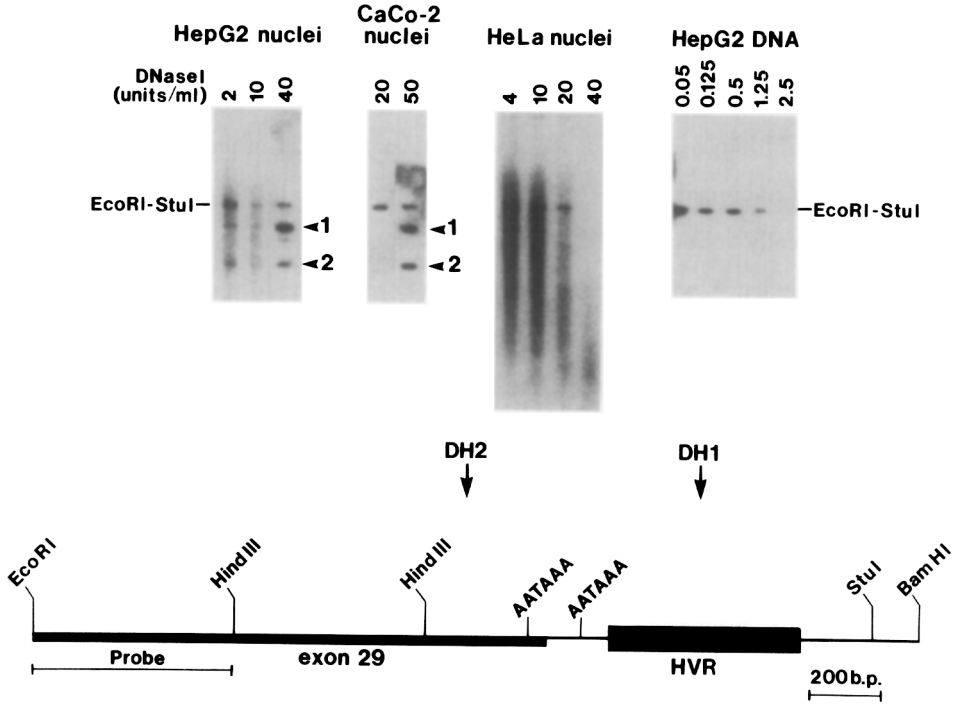

Figure 1. *DNaseI hypersensitive sites in the 3′ end of the human apo-B gene.* Nuclei from HepG2, CaCo-2, and HeLa cells and purified DNA from HepG2 cells were digested with DNase I at the concentration indicated above each lane in the autoradiograms. After digestion, purified DNA from each sample was cleaved with *Eco*RI and *Stu*I, electrophoresed, blotted onto nitrocellulose, and probed with the ^{32}P-labeled probe illustrated schematically in the bottom panel, below the restriction map. DNaseI hypersensitive sites 1 and 2 are indicated on the right side of the autoradiograms in the top panel, and as arrows above the restriction map in the bottom panel. The bottom panel shows the map of the 3′ end of the gene, with exon 29 and the HVR represented by bars. The two AATAAA sequences indicate the positions of the two potential polyadenylation sites of the apo-B gene.

than the 2375 bp *Eco*RI-*Stu*I parent fragment; these bands have been designated as 1 and 2. Band 1 is approximately 1900 bp long, and band 2 is about 1240 bp. The positions of the DH sites 1 and 2 are shown by the arrows above the restriction map in the bottom panel of Figure 1. Four different preparations of nuclei from each of the three cell lines were used in these experiments. The results from two different nuclear preparations are shown for HepG2 cell nuclei; the first two lanes correspond to one batch of nuclei, and the lane at 40 units of DNaseI/ml is derived from a different batch of nuclei. The same two DH sites 1 and 2 were observed in nuclei from CaCo-2 cells (Figure 1, second panel) but not in HeLa cell nuclei (third panel). The absence of any DH sites in free DNA suggests that it is the chromatin organization of that region of the apo-B gene in HepG2 and CaCo-2 cells that permits DNaseI to cut preferentially at sites 1 and 2.

DNaseI hypersensitive site 1 maps in the middle of the A/T-rich HVR. Because of the presence of A/T-rich repeats in the HVR, one would have predicted that this region would be rapidly degraded by DNaseI in a non-selective manner; instead, there was a clear preference for the enzyme to cleave at DH site 1. DNaseI hypersensitive site 2 falls within the 3′ untranslated region of the gene, about 80 bases beyond the translational stop codon. The presence of a hypersensitive site (DH 1) within a nuclear matrix attachment region at the 3′ end of the apo-B gene is reminiscent of the situation at the 3′ end of the chicken lysozyme gene, where DH site 8 falls within the 3′ matrix attachment region region (12).

The apo-B gene 3′ HVR plays a role in anchoring the 3′ end of the gene to the nuclear matrix (8). Furthermore, the region within and flanking DH 1 contains 16 copies of the sequence ATATTT, corresponding to the "invariant core" of the 15-bp topoisomerase II recognition sequence. Therefore, our finding of a DH site in a nuclear matrix attachment region is in good agreement with recent observations of Reitman and Felsenfeld (13) on the chicken β-globin locus; they showed that all strong topoisomerase II recognition sites are found at DH sites. However, not all DH sites are topoisomerase II sites, as exemplified by DH 2 in the apo-B gene.

DNaseI hypersensitive sites have been found at the 3′ ends of a few other genes (2). In most cases they map within 1 kilobase of the polyadenylation signal. The function of some 3′ DH sites appears to be either in transcription enhancement (14), locus activation (15), or transcriptional termination (7). In the case of the apo-B gene, it is tempting to postulate that DH site 2 may play a role in the termination of transcription while DH site 1 may provide an accessible region of DNA for the action of topoisomerase II. If the passage of the transcription complex through the gene generates positive supercoiling ahead of the complex (and

negative supercoiling behind) (13), then topoisomerase II would be required to relax the supercoils. The DH site(s) would facilitate this process. Another possible function for the 3' DH sites described here may be to organize and maintain the apo-B gene domain in a DNaseI sensitive, transcriptionally preactivated state in hepatic and intestinal cells, thus allowing for further modulations by specific cellular signals that affect the transcription of the apo-B gene.

Presence of nucleosomes in the 3' region of the apolipoprotein B gene

It has been suggested that DH sites represent open, accessible regions containing nucleosome-free DNA (16). We were interested in determining whether nucleosomes were

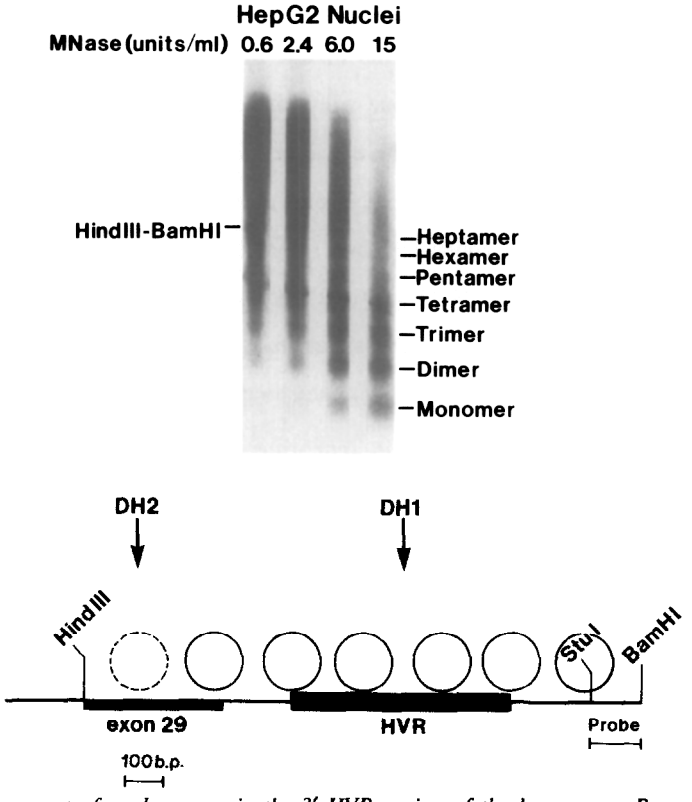

Figure 2. *Presence of nucleosomes in the 3' HVR region of the human apo-B gene.* The top panel shows an autoradiogram of a micrococcal nuclease (MNase) series of digestions of HepG2 cell nuclei followed by secondary digestion of the DNA samples with *Hin*dIII and *Bam*HI. The concentrations of micrococcal nuclease used (in units/ml) is given above the autoradiograms. The position of the parental *Hin*dIII-*Bam*HI band is indicated to the left of the autoradiogram. The position on the gel of the nucleosome monomer and multimers are indicated on the right side. The bottom panel shows the *Hin*dIII to *Bam*HI region, with the exon 29 and HVR portions highlighted by the bars. The location of the probe is shown below the map; DH sites 1 and 2 are indicated by arrows above the map. The tentative positions of the six nucleosomes clearly seen in the autoradiogram are illustrated by solid circles above the map. The location of the seventh nucleosome (broken circle) is not certain because the heptamer band is difficult to see; its positioning in the figure is based on the assumption of the same internucleosomal spacing as for the others.

present in the vicinity of the DH sites at the 3′ end of the apo-B gene, particularly surrounding DH site 1, situated in the matrix attachment region. To this end, we digested nuclei from HepG2, CaCo-2, and HeLa cells with a range of concentrations of micrococcal nuclease, followed by digestion of the purified DNA with *Hin*dIII and *Bam*HI and probing of the Southern blots with an end-labeled fragment (11). The results in Figure 2 show that the 1390-bp *Hin*dIII-*Bam*HI fragment containing the HVR and DH sites 1 and 2 is organized as a regular nucleosomal array in HepG2 cells. The same results were observed in CaCo-2 and HeLa cells (data not shown). The positions of the nucleosomes can be estimated from the relative migration of the monomer, dimer, trimer, and other multimers on the gel, by reference to the position of the *Hin*dIII-*Bam*HI band, and by taking into account the fact that the probe represents the 3′ end of the fragment. Six nucleosomal bands can readily be distinguished in the autoradiogram, enabling us tentatively to position the nucleosomes as shown in the bottom panel of Figure 2. In HepG2 cells, it would appear that DH 1 falls in the linker region between two nucleosomes, while this may not be the case for DH site 2. That DH 1 falls in a topoisomerase II recognition site and that topoisomerase II sites tend to associate with linker DNA (13) add further credence to our preliminary nucleosome localization.

Our data suggest that the 3′ border of the apo-B gene has an ordered or "static" nucleosome organization similar to that observed in the non-transcribed region of the histone H1-H3 gene spacer, in which a matrix anchorage site exists and where nucleosomes are evident in micrococcal nuclease digestions (17). Similarly, the 5′ border of the hsp70 genes, containing nuclear matrix binding sites, is organized into static, phased nucleosomes, and this pattern is maintained during transcriptional activation of these genes (17). The static nucleosome organization may be characteristic of chromatin regions at the border of actively transcribed regions.

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