Volume 13 Number 14 1985 Nucleic Acids Research

Nuclease-hypersensitive sites in chromatin of the estrogen-inducible apoVLDL II gene of chicken

Klaas Kok, Lenie Snippe, Geert AB and Max Gruber

Department of Biochemistry, Groningen University, Nijenborgh 16, 9747 AG Groningen, The Netherlands

Received 19 February 1985; Revised and Accepted 13 June 1985

ABSTRACT

DNAseI-hypersensitive sites were localized in apoVLDL II chromatin from chicken. In the liver two sites at 1.75 and 1.0 kb upstream from the cap-site are present before the gene is activated. After induction by estradiol a number of additional sites appear, three in the promotor region of the gene, one within the coding region and two behind the poly-A signal. These sites disappear when the expression of the gene is shut off upon estradiol withdrawal. All sites appear to be tissue-specific in that they are not found in other tissues of the rooster. However, in oviduct of the laying hen we find a hypersensitive site at 1.6 kb in front of the gene.

INTRODUCTION

After the discovery of the nucleosomal structure of chromatin (1,2), attempts have been made to find differences between active and inactive chromatin. Nucleases have proven to be useful tools in the analysis of chromatin structure (3,4). Transcriptionally active genes, and in some cases potentially active genes as well, turned out to be preferentially susceptible to the nucleolytic action of DNAseI and micrococcal nuclease (5-8). In addition to the general nuclease sensitivity of larger DNA segments, narrow regions with a markedly higher succeptibility to nucleases are found. Most often DNAseI has been used(3,9-12), but hypersensitive sites have also been found with undefined endogenous nucleases (8,11), micrococcal nuclease (12-14) and S-1 nuclease (15).

Many hypersensitive sites appear to be tissue specific (16-18). Often, these hypersensitive sites are found within 1 kb in front of genes that are active or can be activated in the tissue studied (10,18-21). The local chromatin structure that is responsible for the DNAseI-hypersensitivity is not yet clear. Some evidence points towards a stretch of DNA which is free of nucleosomes (22), and where other proteins may bind (23-26). These proteins might be involved in the control of gene expression (24,26). The nonhistone chromatin proteins HMG 14 and 17 are thought to be involved in the

general nuclease sensitivity of transcribed genes (27).

In this paper, we describe the DNAseI sensitivity of the chicken apo Very Low Density Lipoprotein II (apoVLDL II) gene region and the localization of nuclease hypersensitive sites. The apoVLDL II gene codes for a yolk protein. Normally this gene is only expressed in laying hen livers, but estrogen administration to roosters leads to its transcription in rooster liver (28). We thus have the possibility to study the tissue specificity before and after gene activation. Moreover, the reversibility of the hormone effect allows us to study changes after deactivation (29).

MATERIALS AND METHODS

Animals

White Leghorn roosters and hens were obtained from the poultry hatchery Van Der Sterren, Venray, the Netherlands. Roosters were injected subcutaneously with 50 mg diethylstilbestrol (Sigma, St. Louis, MO, USA) per kg body weight. The roosters were 6 to 10 weeks old, unless otherwise stated.

Nuclei

Nuclei were isolated according to the method of Hewish and Burgoyne (30), using a homogenisation buffer containing 0.35 M sucrose, 25 mM KCl, 5mM $MgCl_2$, 1 mM EGTA, 0.5 mM spermine, 0.15 mM spermidine, 0.1 mM PMSF and 50 mM Tris-HCl pH 7.5. Tissues were briefly homogenized with an ultra turrax (Janke & Kunkel GmbH, Staufen, FRG) before douncing with a glass grinder. Nuclei from liver and oviduct were collected by centrifugation through a 1.9 M sucrose layer. Heart nuclei were centrifuged through 1.5 M sucrose. Erythrocytes were lysed as described by Leake et al.(31), and the nuclei were centrifuged through 1.9 M sucrose. All isolated nuclei were washed once with the DNAseI digestion buffer containing 0.35 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.1 mM PMSF, 10 mM Tris-HCl pH 7.5, and resuspended at a DNA concentration of 1 mg/ml.

Nuclease digestions

DNAseI digestion. Nuclei were preincubated for two min at 37°C and digested with 0.1 to 12.8 U DNAseI (Worthington, Freehold, N.J., USA) per ml at 37°C for 10 min. The reaction was stopped by addition of an equal volume of lysis buffer containing 1% SDS, 0.3 M NaCl, 20 mM EDTA, 10 mM EGTA, pH 7.5, and heating for two min at 60°C.

Micrococcal nuclease digestion. Nuclei were digested with 15 U micrococcal nuclease (Worthington) per ml for 0.5 to 8 min at 37°C in the DNAseI digestion buffer supplemented with 0.4 mM $CaCl_2$. The reaction was terminated as above.

Endogenous nuclease digestion. Nuclear preparations were autodigested at 37°C for intervals up to 60 min in the micrococcal nuclease digestion buffer.

DNA purification

The lysed nuclei were incubated with 50 µg proteinase K per ml for 2 h at 37°C and then for 16 h at room temperature. The DNA was deproteinized by two phenol extractions, one phenol-chloroform-isoamyl alcohol (25:24:1 v/v) extraction and one chloroform-isoamyl alcohol (24:1 v/v) extraction. The water layer was extracted twice with ether, treated for 1 h at 37°C with 50 U pancreatic RNAse (DNAse-free) per ml, and 16 h at room temperature with 50 µg proteinase K per ml, and again deproteinized as before. The water layer was extracted twice with butanol-2. The DNA was precipitated with 2.5 volumes of ethanol and dissolved in 1 mM EDTA, 10 mM Tris-HCl pH 7.8, to a concentration of 0.2 to 1 mg/ml. To check whether the chromatin was properly digested with the nuclease used, 2-µg samples of the DNA were electrophoresed on a 0.7% agarose gel.

Restriction and blotting analysis of genomic DNA

Restriction enzymes were purchased from Boehringer (Mannheim, FRG). Incubation conditions were as described by the manufacturers. The enzymes were used at 3 to 5 units per µg DNA and incubated for at least 5 hours. The digested DNA was purified by one phenol extraction followed by one extraction with chloroform. The water layer was extracted twice with butanol-2 and twice with ether. Samples of 10 to 20 µg of DNA were applied to a 1% agarose gel; electrophoresis was for 16 h at 2 V/cm. The DNA was transferred to a BA 85 nitrocellulose filter (Schleicher & Schüll, Dassel, FRG), and hybridized essentially as described (32,33). The filters were autoradiographed for 2 to 8 days on Kodak XAR-5 film, using an intensifying screen (Cronex, Dupont, Wilminton, DE, USA) at -80°C.

Routinely, EcoRI and HindIII-digests of lambda phage DNA were used as size markers, together with a Sau3AI-digest of pBR329. In some experiments a partial Sau3AI-digest of the 5.5-kb EcoRI fragment of the apoVLDL II gene, combined with a HinfI- and a BstEII-digest of the same fragment, was used as a size marker, in which case the marker was blotted and hybridized along with the chromatin samples.

Probes

Small restriction fragments were subcloned in M13mp8 and M13mp9 (34). Labelled probes were synthesized with Klenow polymerase and [α-^{32}P]dCTP, using single-stranded phage DNA as template (35). The ovalbumin probe, a 9.5-kb HpaII-HhaI fragment covering the entire gene (6), was a gift of

Prof.Dr.P. Chambon (Strasbourg, France). This probe was labelled by nick translation.

RESULTS

DNAseI-sensitivity of the apoVLDL II gene

The sensitivity of the apoVLDL II gene in chromatin towards DNAseI was investigated by monitoring the concentration of two EcoRI fragments covering the entire apoVLDL II gene (Fig. 1). Southern blots of EcoRI restricted DNA from the DNAseI treated chromatin, were hybridized with a probe specific for the apoVLDL II gene. The simultaneous addition of an ovalbumin probe allowed us to compare the sensitivities of the apoVLDL II gene and the ovalbumin gene in one experiment (Fig. 2). The expression of the ovalbumin gene is regulated by the same hormone, but the gene is transcribed only in the oviduct (6-8).

There is no difference in DNAseI sensitivity between the apoVLDL II gene and the ovalbumin gene in heart nuclei (panel A) and erythrocyte nuclei (panel B). The same holds true for liver nuclei of the untreated rooster (panel D), at least for the 3.9-kb apoVLDL II fragment. However, the 5.5-kb apoVLDL II fragment appears slightly more sensitive than the 3.9-kb fragment. In liver nuclei from roosters 72 h after estrogen administration (panel E) and from laying hen (panel F), both apoVLDL II fragments are more sensitive towards DNAseI digestion than the ovalbumin fragments. The situation is completely different for the oviduct nuclei of a laying hen (panel C). Here, in comparision to the apoVLDL II gene, the ovalbumin gene appears more sensitive. This result is in good agreement with the finding that an actively trans-

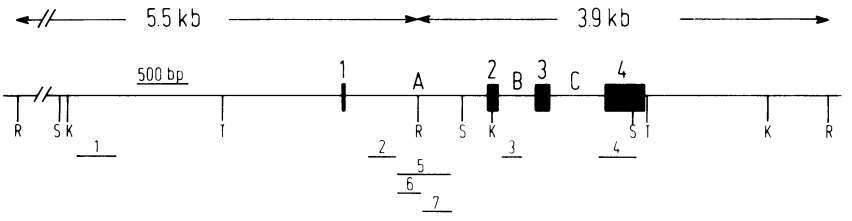

Fig. 1. Map of the apoVLDL II gene. Above the map, the exons (1-4), the introns (A, B, C) and the 5.5- and 3.9-kb EcoRI fragments are indicated. Below the map, the relevant restriction sites (R=EcoRI, K=KpnI, S=SacI, T=TaqI) are indicated, as well as the positions of the probes: a 376-bp Sau3AI fragment (probe 1); a 283-bp PstI fragment (probe 2); a 206-bp AluI fragment (probe 3); a 348-bp Sau3AI fragment (probe 4); a 510-bp PstI fragment (probe 5); a 239-bp PstIxEcoRI fragment (probe 6) and a 271-bp EcoRIxPstI fragment (probe 7).

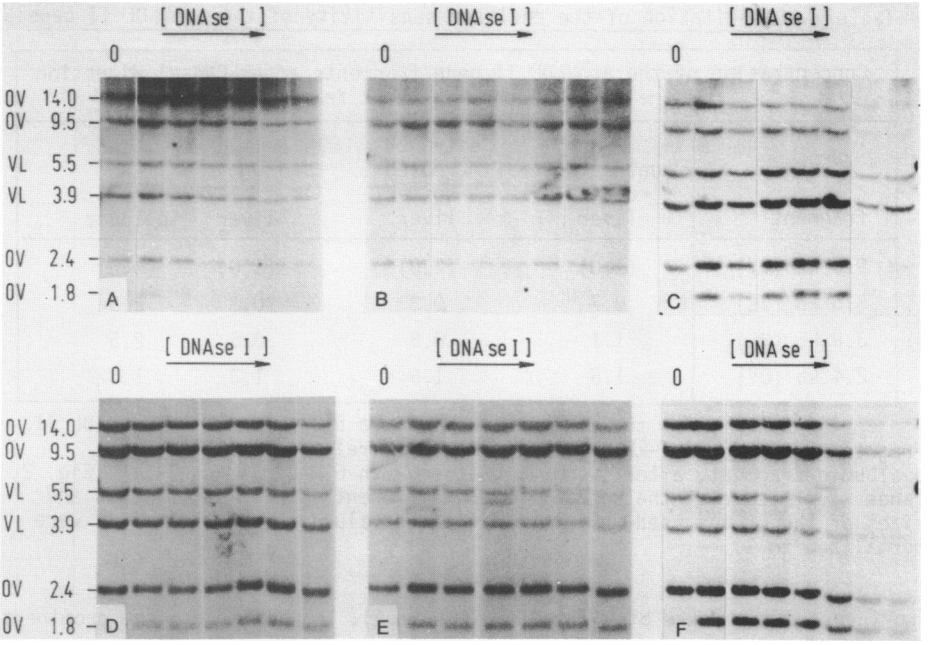

Fig. 2. DNAseI-sensitivity of the apoVLDL II gene region. Nuclei from heart (A), erythrocytes (B), oviduct (C), untreated rooster liver (D), estrogen treated rooster liver (E) and hen liver (F), were treated with increasing DNAseI concentrations (0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 9.6 U/ml). The 5.5- and 3.9-kb EcoRI fragments of the apoVLDL II gene, and the 14.0-, 9.5-, 2.4- and 1.8-kb EcoRI fragments of the ovalbumin gene were visualized by hybridization of Southern blots with apoVLDL II probe 5 (see Fig. 1) and an ovalbumin probe, respectively.

cribed gene is more sensitive towards DNAseI digestion than an inactive gene.

To obtain a more objective estimate of the intensities of the bands, the autoradiograms, where necessary different exposures of the same blot, were scanned. The results, of which the most relevant data are compiled in Table I, confirm the conclusions made by visual examination of the autoradiograms.

DNAseI-hypersensitive sites in the apoVLDL II gene region

Liver nuclei from control and 72-h estrogen-treated roosters were investigated for the presence of DNAseI-hypersensitive sites in the apoVLDL II gene region. Nuclei were mildly digested with increasing amounts of DNAseI. The purified DNA was subsequently cut to completion with EcoRI, and the DNA fragments were separated on a 1% agarose gel. The DNA was transferred to a nitrocellulose filter and hybridized following the indirect end-labelling method (10) with one of two short probes adjacent to either side of the

Table I. Quantitation of the nuclease sensitivity of the apoVLDL II gene.

Concentration of the apoVLDL II gene fragments after DNAseI digestion (relative to the 9.5-kb OV fragment)				
fragment	untreated rooster liver	estrogen-treated rooster liver	laying hen liver	laying hen oviduct
9.5 kb (OV)	(1.0)	(1.0)	(1.0)	(1.0)
5.5 kb (VL)	0.7	0.3	0.3	2.5
3.9 kb (VL)	1.1	0.6	0.5	2.5
2.4 kb (OV)	1.5	1.6	1.3	1.6

The table gives the concentration of the 5.5- and 3.9-kb apoVLDL II fragments, and the 2.4-kb ovalbumin fragment, relative to the 9.5-kb ovalbumin fragment, after 10 min digestion with 6.4 U/ml DNAseI (see Fig. 2, lanes 7). To correct the signal for the different specific activities and sizes of the probes used, all zero-digestion values (Fig. 2, lanes 1) were normalized to 1.

EcoRI restriction site at 0.78 kb (in intron A). In addition to the prominent bands, representing the 5.5-kb or 3.9-kb EcoRI restriction fragment, a number of weaker bands appear (Fig. 3). These bands must result from cleavage of the chromatin by DNAseI at specific sites. The appearance of these bands depends

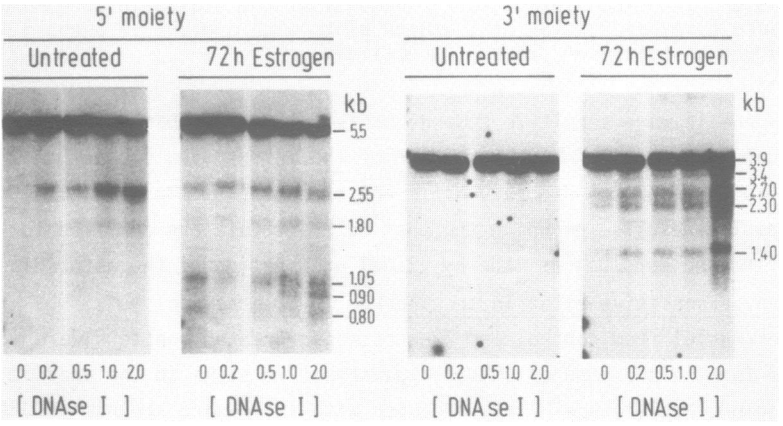

Fig. 3. DNAseI-hypersensitive sites of apoVLDL II chromatin. Liver nuclei from untreated roosters and estrogen treated roosters were digested with DNAseI at the concentrations indicated (U/ml). Southern blots of the EcoRI-restricted DNA were hybridized with probes 2 and 3 (see Fig. 1) for the 5'- and 3'-moiety of the gene region, respectively.

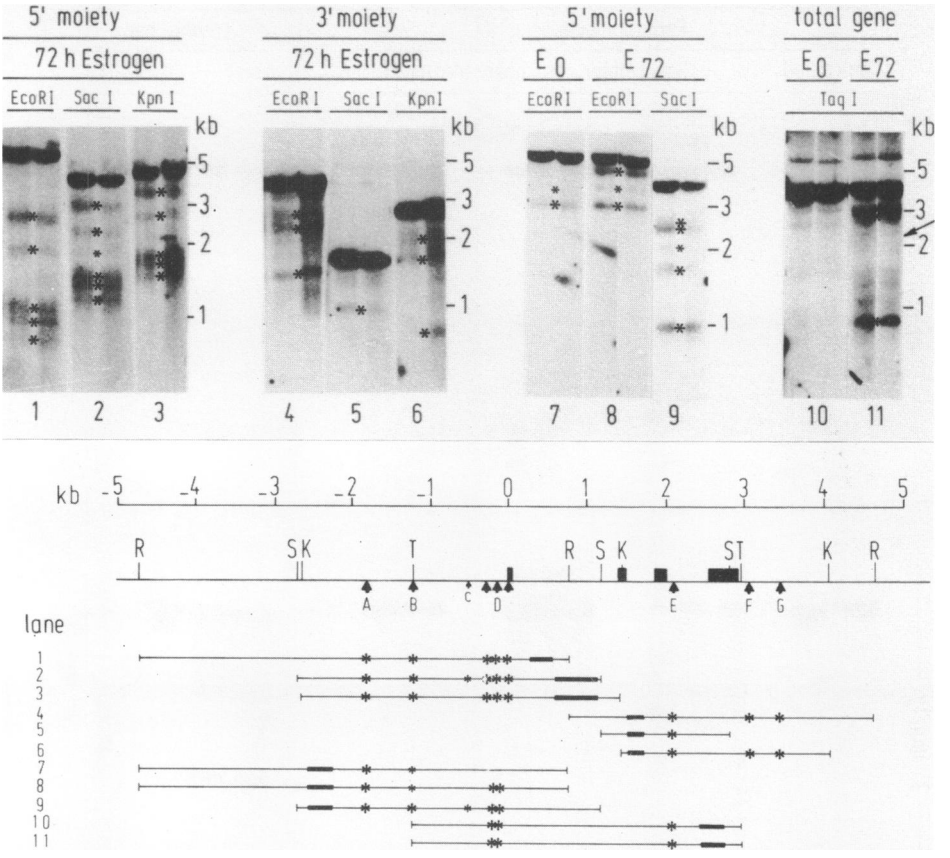

Fig. 4. Mapping of DNAseI-hypersensitive sites with different probes. Liver nuclei from untreated roosters (E_0) and 72-h estrogen treated roosters (E_{72}) were digested with DNAseI (0.8 and 1.6 U/ml). Southern blots of the DNA, restricted as indicated, were hybridized with various probes, of which the positions are given as solid bars in the schematic drawing. For each lane the investigated restriction fragment is shown as a thin line. The asterisks correspond to the asterisks in the autoradiographs, and indicate the positions of the hypersensitive sites. In the map, the hypersensitive sites (A to G), the restriction sites (R=EcoRI, K=KpnI, T=TaqI, S=SacI) and the apoVLDL II exons (black bars) are indicated.

upon the presence of nuclease, since their intensity increases upon prolonged DNAseI treatment. The bands occasionally present in the controls, incubated without DNAseI are caused by the action of endogenous nucleases (see below).

In the 5'-moiety of the gene region, two hypersensitive sites are found in the untreated liver (Fig. 3). The bands of 2.55 kb and 1.8 kb correspond to sites at 1.75 and 1.0 kb upstream from the first exon. These sites are

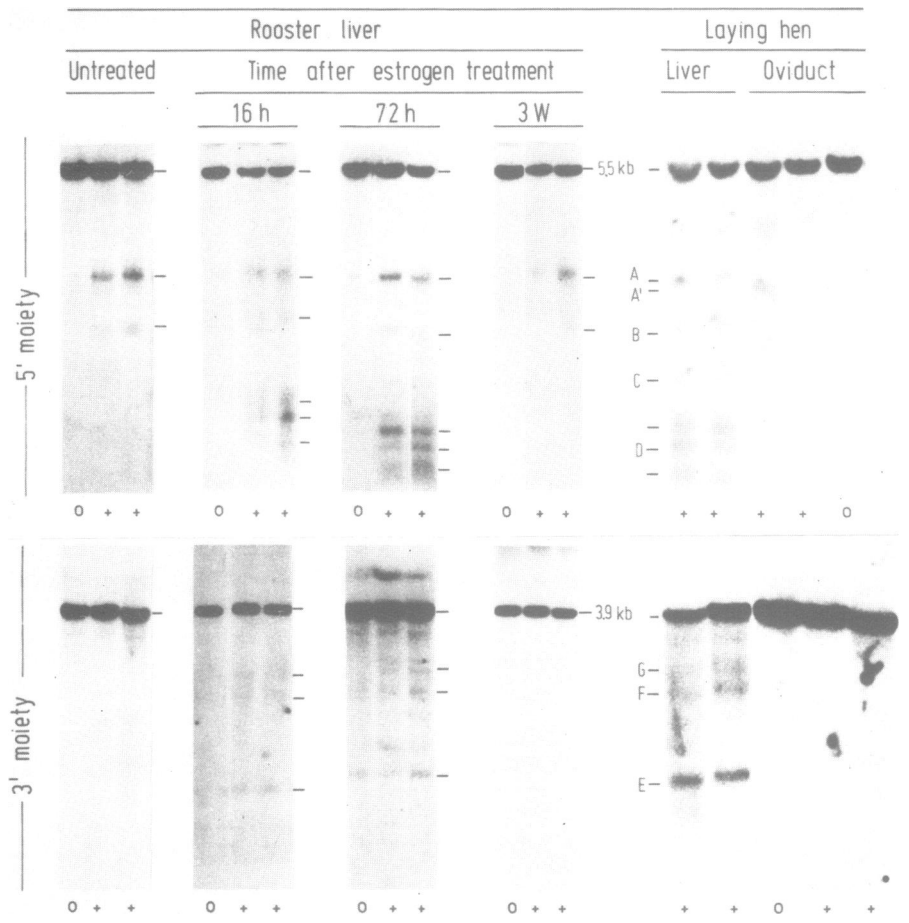

Fig. 5. Estrogen dependence of hypersensitive sites of apoVLDL II chromatin. Nuclei from the livers of untreated roosters and roosters at 16 h, 72 h, or 3 weeks after a single dose of estrogen, and nuclei from the liver and oviduct of hens were incubated with (+) or without (o) DNAseI. Southern blots of EcoRI-restricted DNA were hybridized with probes 6 and 7 (see Fig. 1) for the 5'- and 3'-moiety of the apoVLDL II gene region, respectively. The lettering of the hypersensitive sites in liver refers to the map in Fig. 4. The hypersensitive site in oviduct is indicated by A'.

also observed after stimulation with estrogen, but in addition three new sites appear, mapping within 300 bp upstream from the cap-site. Occasionally, a weak hypersensitive site can be seen, lying 500 bp upstream from the cap-site (site C, see Fig. 4). The same EcoRI digests were probed for DNAseI-hypersensitive sites within the 3.9-kb EcoRI fragment containing the 3'-moiety of the gene (Fig. 3). In liver nuclei of the untreated rooster only the

3.9-kb fragment can be seen, indicating that in this region no hypersensitive sites are present. Three days after a single hormone dose, a number of hypersensitive sites have appeared. One site maps about 1.4 kb downstream from the EcoRI site and lies in intron C. Two other sites (bands of 2.3 and 2.7 kb in Fig. 3) map 150 and 550 bp downstream from the poly A-site. Under optimal conditions of separation, these bands are resolved as doublets (see Fig. 5). The position of these DNAseI-hypersensitive sites was confirmed by the use of two other restriction enzymes, KpnI and SacI. The results for the estrogen-treated liver nuclei are shown in Fig. 4 (lanes 1 to 6). They are in good agreement with the results obtained with the EcoRI digestions (Fig. 3). Sites mapping at the same position were found irrespective of the enzyme used. With KpnI and SacI digests of DNA from liver nuclei of untreated roosters only the hypersensitive sites at 1.75 and 1.0 kb in front of the gene are found (data not shown).

The estradiol-dependent site corresponding to the 3.4-kb band in the EcoRI digest (Fig. 3) cannot be mapped unambiguously. This is due to the position of the probe (probe 3 in Fig. 1) used, which is at some distance from the EcoRI site. Instead of indicating a hypersensitive site downstream from the poly A-signal, the 3.4-kb band most likely corresponds to a set of weak hypersensitive sites around map position 1.4 to 2.0 kb observed in a TaqI digest (see below, Fig. 4).

In the analysis of the 5'-moiety, the Southern blots were also hybridized with a second probe, situated at the distal side of the SacI restriction fragment (Fig. 4, lanes 7-9). Again the hypersensitive sites map at the same positions. Due to the presence of repetitive DNA (36), probes at the distal side of the 5.5-kb EcoRI fragment could not be used.

In the experiments discussed so far, DNAseI-hypersensitive sites around the EcoRI site in intron A might easily have been overlooked. Therefore, TaqI digests of DNAseI treated chromatin were used to detect such sites. The whole apoVLDL II gene, including 1.1 kb of the 5'-flanking DNA, is contained in one large TaqI fragment. The results are shown in Fig. 4 (lanes 10 and 11). The arrow indicates the position of the EcoRI site within the TaqI fragment. One may conclude that there are no major hypersensitive sites between the site just in front of the gene and the site in the third intron of the apoVLDL II gene, although a few weak bands can be seen, which are estradiol dependent.

In the experiments shown, the EcoRI digests were hybridized with probes lying a few hundred base pairs from the EcoRI site. However, similar results

were obtained when EcoRI-PstI fragments abutting the EcoRI site in intron A were used as probes (see also Fig. 5).

The hypersensitive sites discussed above are also apparent when endogenous nucleases present in the liver (8,11,30) are allowed to act (data not shown). Mild digestion of nuclei with micrococcal nuclease again revealed the same set of hypersensitive sites (data not shown), despite the known sequence specificity of this nuclease (38,39). Moreover, both nucleases reveal the same changes in chromatin structure upon estrogen treatment, as does DNAseI.

The inducible hypersensitive sites are independent of DNA-replication and are reversible

Transcription of the apoVLDL II gene starts within 1 h after a single dose of estrogen. Between one and three days after hormone administration, the rate of apoVLDL II precursor-mRNA synthesis is at its maximum, after which it decreases rapidly (M. Noteborn, personal communications). After ten days, no more apoVLDL II precursor mRNA can be detected in the liver cells, indicating that the gene is no longer expressed. A concomitant response to estrogen is a 35% increase of the DNA content of the liver, that occurs between 24 and 32 hours after hormonal induction (37).

To investigate whether the estrogen-dependent hypersensitive sites appear before the DNA is replicated, liver nuclei were isolated 16 h after a single injection of estrogen. At this stage, the same DNAseI-hypersensitive sites are found as after 72 h (Fig. 5). Apparently, the changes in chromatin structure take place before DNA replication is started.

Of the additional bands in the 3'-moiety observed in the estrogen-treated liver, the 2.9-kb band represents another hypersensitive site lying close to site G, which is not always resolved as a separate band. The 3.4-kb band observed in the 72-h estrogen sample is not consistently found. Its appearance in the untreated liver sample indicates that it does not correspond to the 3.4-kb band discussed in Fig. 3, which is estradiol-dependent. Moreover, the 16-h rooster liver (Fig. 5) does not contain a band at this position, nor does the hormone-withdrawn liver. Maybe, the band originates from a different region of the genome showing some cross-hybridization with the probe used.

Probing liver nuclei of roosters three weeks after a single injection of estrogen for DNAseI-hypersensitive sites, shows that the occurence of these sites is reversible (Fig. 5). Only the hormone-independent sites, positioned at 1.75 and 1.0 kb upstream from the cap-site, are still present. There is no difference in the position and the number of DNAseI-hypersensitive sites between liver nuclei from hormone-withdrawn roosters and control roosters.

DNAseI-hypersensitive sites in the liver and the oviduct of laying hen

The hypersensitive sites in apoVLDL II chromatin described above for rooster liver are not present in heart tissue and erythrocytes (data not shown). To investigate the tissue specificity of these sites further, we compared the liver and the oviduct of laying hens. Both tissues are targets for estradiol, but only the liver responds with the expression of the apoVLDL II gene. The liver of the laying hen contains the same set of hypersensitive sites in the apoVLDL II gene region as the liver of the estrogen-treated rooster (Fig. 5). For the oviduct the picture is different. No hypersensitive sites are present in the regions where estradiol administration induces such sites in liver chromatin. Further upstream where liver contains site A (at -1.75 kb), the oviduct also has a prominent hypersensitive site. Closer examination of these sites shows that their positions are distinctly different, the site in oviduct (A'; see Fig. 5) lying about 150 nucleotides closer to the cap-site. The subband which migrates between the 5.5-kb parent band and the "A" band was only present in a few blots and not further investigated.

DISCUSSION

As a first approach, we investigated the overall nuclease sensitivity of the apoVLDL II gene region in different tissues, and compared it to that of the ovalbumin gene. In the rooster liver, the apoVLDL II gene, but not the ovalbumin gene is potentially active. Nevertheless, there is no difference in the overall DNAseI sensitivity between the ovalbumin gene fragments and the 3.9-kb fragment covering the larger part of the apoVLDL II gene. Only upon induction of its expression by estradiol administration, the apoVLDL II gene in the liver obtains increased sensitivity for DNAseI.

In comparing the sensitivities of different parts of the apoVLDL II chromatin in untreated roosters, we observed that the 5'-moiety contained in the 5.5-kb EcoRI fragment is slightly more sensitive towards DNAseI attack than the 3.9-kb EcoRI fragment containing the 3'-moiety of the gene. Possibly, the enhanced sensitivity of the 5'-moiety over the 3'-moiety can be explained by the presence of hypersensitive sites exclusively in the former region.

We detected three DNAseI-hypersensitive sites lying within 300 bp upstream from the cap-site, which are only present when the gene is transcribed. This region is also hypersensitive towards other nucleases, namely micrococcal nuclease and an endogenous nuclease, and to the chemical agent methidium propyl-EDTA (40, data not shown). These DNAseI sites appear within 16 hours

after a single dose of estrogen, and disappear again after hormone withdrawal. A similar close relationship between gene expression and the presence of hypersensitive sites in the 5'-flanking DNA has been found for a number of other genes, including the lysozyme gene in chicken oviduct (11,41), the thymidine kinase gene in mouse liver (21), the histone genes in sea urchin embryos (19), and the tyrosine amino transferase gene in rat liver (17). It has been suggested that this hypersensitivity is a direct manifestation of the altered chromatin structure that makes the promotor region accessible to the enzyme RNA polymerase (19). However, these expression-linked sites are not always found in the immediate vicinity of the promotor region. For the tyrosine amino transferase gene (17), the inducible site lies 2 kb upstream from the promotor. Furthermore, we (unpublished results) and others (20) have mapped two DNAseI-hypersensitive sites in front of the vitellogenin gene that appear after induction of the gene, but persist at least for some weeks after hormone withdrawal. Whether the sites we find within and behind the transcribed region are involved in the activation, or just a consequence of the transcription process, cannot be decided yet.

Two other DNAseI-hypersensitive sites lying 1.75 and 1.0 kb upstream from the apoVLDL II gene are always present in the rooster liver. They are not found in heart nuclei, in erythrocytes and in naked DNA, and are thus tissue specific and not expression specific. Tissue specific hypersensitive sites have also been found for the tryptophan oxygenase gene and the tyrosine amino transferase gene of rat liver (17) and the glue protein gene Sgs-4 of the salivary gland cells of Drosophila (16). Sites specific for estrogen-responsive tissues have been observed within the chicken vitellogenin gene and its 3'-flanking region (20). A number of sites found in front of the chicken ovalbumin gene in the oviduct are not present in erythrocytes, but a clear distinction between tissue specificity and induction specificity has not been made yet (22). The same holds true for the β-globin gene, although a study of Friend erythroleukemia cells revealed the presence of a hypersensitive site before the gene was activated (42).

Surprisingly, in the oviduct of the laying hen, we found a DNAseI-hypersensitive site at 1.6 kb upstream from the cap-site. Thus, there is a clear difference in the chromatin structure of the apoVLDL II gene between the oviduct on the one hand, and heart and erythrocytes on the other. The hypersensitive site in the oviduct lies close to the tissue-specific site found in the rooster liver, the difference being about 150 bp. We speculate that the chromatin structure of this region may be crucial in determining the

tissue specificity of apoVLDL II expression.

The structural basis of hypersensitive sites is not known. Current evidence suggests that the nucleosomal structure is interrupted around the hypersensitive sites, creating a stretch of nucleosome-free DNA. Such a region has been shown to exist for the SV40 early promotor region (22), and might also be present just in front of the β-globin gene (12). A number of publications report the finding of non-histone proteins that bind in the vicinity of the DNAseI-hypersensitive sites (23-26). These proteins will presumably bind at specific sequences, and may be responsible for the absence of nucleosomes.

The apoVLDL II gene and 2.5-kb of the 5'-flanking region has been sequenced in our laboratory (43, and R. Strijker, personal communications). No striking sequence homologies were found between the different DNAseI-hypersensitive sites within the apoVLDL II gene region, nor between these sites and those of the vitellogenin gene described by Burch (20,44). The symmetrical protein recognition sequence described by Borgmeyer et al.(25), mapping close to a DNAseI-hypersensitive site in front of the lysozyme gene (25) and the c-myc gene (26) was not found in the vicinity of the apoVLDL II gene. So, it remains to be determined, which sequences and/or protein interactions are responsible for the DNAseI-hypersensitive sites within the apoVLDL II gene region.

ACKNOWLEDGEMENTS

Special thanks are given to Prof.Dr.P. Chambon (Strasbourg, France) for kindly sending us the ovalbumin plasmid. We also thank mr.J. Bouwer for skilful assistance in animal experiments, mr.N. Panman for assistance in preparing the figures, and mr.K. Gilissen for photography. This investigation was carried out with financial aid of the Netherlands Organization for the Advancement of pure research (Z.W.O.) and the Netherlands foundation for chemical research (S.O.N.).

REFERENCES

1. Kornberg, R.D. (1974), Science 184, 868-871.
2. Kornberg, R.D. (1977), Annu. Rev. Bioch. 46, 931-954.
3. Elgin, S.C.R. (1981), Cell 27, 413-415.
4. Reeves, R. (1984), Biochim. Biophys. Acta 782, 343-393.
5. Weintraub, H. and Groudine, M. (1976), Science 193, 848-856.
6. Bellard, M., Kuo, M.T., Dretzen, G. and Chambon, P. (1980), Nucleic acids Res. 8, 2737-2750.
7. Lawson, G.M., Knoll, B.J., March, C.J., Woo, S.L.C., Tsai, M. and O'Malley, B.W. (1982), J. Biol. Chem. 257, 1501-1507.
8. Anderson, J.N., Vanderbilt, J.N., Lawson, G.M., Tsai, M. and O'Malley, B.W. (1983), Biochemistry 22, 21-30.

9. Stalder, J., Larsen, A., Engel, J.D., Dolan, M., Groudine, M. and Weintraub, H. (1980), Cell 20, 451-460.
10. Wu, C. (1980), Nature 286, 854-860.
11. Fritton, H.P., Sippel, A.E. and Igo-Kemenes, T. (1983), Nature 11, 3469-3485.
12 McGhee, J.D., Wood, W.I., Dolan, M., Engel, J.D. and Felsenfeld, G. (1981), Cell 27, 45-55.
13. Keene, M.A. and Elgin, S.C.R. (1981), Cell 27, 57-64.
14. Kaye,J.S., Bellard, M., Dretzen, G., Bellard, F. and Chambon, P. (1984), EMBO J. 3, 1137-1144.
15. Larsen, A. and Weintraub, H. (1982), Cell 29, 609-622.
16. Shermoen, A.W. and Beckendorf, S. (1982), Cell 29, 601-607.
17. Becker, P., Renkawitz, R. and Schütz, G. (1984). EMBO J. 3, 2015-2020.
18. Keene, M.A., Corces, V., Lowenhaupt, K. and Elgin, S.C.R. (1981), Proc. Natl. Acad. Sci. USA 78, 143-146.
19. Bryan, P.N., Olah, J. and Birnstiel, M.L. (1983), Cell 33, 843-848.
20. Burch, J.B.E. and Weintraub, H. (1983). Cell 33, 65-76.
21. Sweet, R.W., Chao, M.V. and Axel, R. (1982), Cell 31, 347-353.
22. Jongstra, J., Reudelhuber, T.L., Oudet, P., Benoist, C., Chae, C., Jeltsch, J., Mathis, D.J. and Chambon, P. (1984), Nature 307, 708-714.
23. Emerson, B.M. and Felsenfeld, G. (1984), Proc. Natl. Acad. Sci. USA 81, 95-99
24. Wu, C. (1984), Nature 309, 229-234.
25. Borgmeyer, U., Nowock, J. and Sippel, A.E. (1984), Nucleic Acid Res. 12, 4295-4311.
26. Siebenlist, U., Henninghausen, L., Battey, J. and Leder, P. (1984), Cell 37, 381-391.
27. Weisbrod, S. and Weintraub, H. (1979), Proc. Natl. Acad. Sci. USA 76, 630-634.
28. Chan, L., Means, A.R. and O'Malley, B.W. (1978) in Vitamins and Hormones 36, 259-290.
29. Deeley, R.G. and Goldberger, R.F. (1979) in Ontogeny of receptors and reproductive hormone action pp. 291-307, eds. Hamilton, T.H., Clark, J.H. and Sadler W.A., Raven Press, New York.
30. Hewish, D.R. and Burgoyne, L.A. (1973). Bioch. Biophys. Res. Commun. 52, 504-510.
31. Leake, R.E., Trench, M.E. and Barry, J.M. (1972), Exptl.Cell. Res.71,17-26.
32. Southern, E.M. (1975), J. Mol. Biol. 98, 503-517.
33. Jeffreys, A.J. and Flavell, R.A. (1977), Cell 12, 429-439.
34. Messing, J. and Vieira, J. (1982), Gene 19, 269-276.
35. Sanger, F.., Nicklen, S. and Coulson, A.R. (1977), Proc. Natl. Acad. Sci. USA 74, 5463-5467.
36. Meijlink, F.C.P.W. (1983), Ph.D. Thesis, The University of Groningen.
37. van den Berg, J.A., Kooistra, T., AB, G. and Gruber, M. (1974), Bioch. Bioph. Res. Commun. 61, 367-374.
38. Horz, W. and Altenburger, W. (1981), Nucleic Acid Res. 9, 2643-2658.
39. Dingwall, C., Lomonossoff, G.P. and Laskey, R.A. (1981), Nucleic Acids Res. 9, 2659-2673
40. Cartwright, I.L., Herzberg, R.P., Dervan, P.B. and Elgin, S.C.R. (1983), Proc. Natl. Acad. Sci. USA 80, 3213-3217.
41. Fritton, H.P., Igo-Kemenes, T., Nowock, J., Strech-Jurk, U., Theisen, M. and Sippel, A.E. (1984), Nature 311, 163-165.
42. Balcarek, J.M. and McMorris, F.A. (1983), J. Biol. Chem. 258,10622-10628.
43. van het Schip, A.D., Meijlink, F.C.P.W., Strijker, R., Gruber, M., van Vliet, A.J., van de Klundert, J.A.M. and AB, G. (1983), Nucleic Acids Res. 11, 2529-2540.
44. Burch, J.B.E. (1984), Nucleic Acids Res. 12, 1117-1135.