The DNase I sensitive domain of the chicken lysozyme gene spans 24 kb

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ABSTRACT

We have determined the DNase I sensitive chromatin domain of the lysozyme gene in the hen oviduct. When nuclei were digested with DNase I, about 14 kb of upstream and 6 kb of downstream sequences in addition to the 4 kb long transcribed region were preferentially degraded. The transcription start site is located near the center of the approximately 24 kb long sensitive domain. At the 3' boundary there is a rather abrupt transition from the DNase I sensitive to the resistant chromatin configuration whereas at the 5' border this transition occurs in a gradual fashion over 6-7 kb of DNA. No obvious correlation between the boundaries of the domain and repetitive sequences could be established. DNase I-hypersensitive sites are clustered within the boundaries of the sensitive domain which seems to represent a functional unit of the gene.

INTRODUCTION

The expression of eukaryotic genes is controlled in part from remote sites that can be thousands of base pairs (bp) away from the gene. This raises the question of how the boundaries of eukaryotic genes can be defined? Investigation of the chromatin structure of active genes is a possible approach towards answering this question, since active genes are known to have an altered chromatin conformation that is preferentially sensitive to digestion with DNase I (reviews: 1-4). This sensitivity is not restricted to the coding region itself, but extends far upstream and downstream from genes, as well (e.g. 5-7). It is conceivable that the sensitive chromatin domain represents the functional unit of a gene. Lawson et al. (8) showed that the chicken ovalbumin and its related genes, X and Y, exist in a 100 kb DNase I-sensitive domain in the oviduct. On the other hand the DNase I sensitive domain of the gene for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is only 12 kb long (9). The β -globin gene family in murine erythroleukemia cells resides within two distinct chromatin domains differing in their DNase I sensitivity (10). Which of the two domains is in a sensitive chromatin conformation depends on the functional state of the cell.

A different approach for the determination of chromatin domains was used by others. Recent observations have pointed to the possibility that active genes are under torsional stress (e.g. 11). Maintenance of torsional stress requires that the chromatin fiber is attached to a supporting framework of the nucleus giving rise to chromatin loops (domains). Specific scaffold or matrix attachment sites were characterized around the histone and heat shock genes of *Drosophila* (12) and a kappa immunoglobulin gene of the mouse (13). However, the relationship between DNase I sensitive domains and domains that are defined as the region between two matrix attachment sites is not yet clear.

We have been trying to understand the functional relationship between chromatin structure and tissue specific activation of the lysozyme gene of the hen. In previous work we examined the chromatin structure of this gene in hen oviduct and erythrocytes for the presence of DNase I-hypersensitive sites (14). Some of these sites turned out to be targets for in vitro binding of effectors of transcription (15,16) and for action of steroid hormones (17) emphasizing the relevance of DNase I-hypersensitive sites as control elements in eukaryotic gene regulation.

The aim of the present work was to establish the size of the domain of the lysozyme gene in the hen oviduct by determining the sensitivity to DNase I in and around the transcription unit. For this it was necessary to extend previous cloning work (18,19) by "chromosomal walking" experiments, to refine and extend established restriction maps, and to localize repetitive DNA sequences in the newly cloned regions in order to avoid problems when using fragments as hybridization probes. We also investigated the new regions for their presence of DNase I-hypersensitive sites.

MATERIALS AND METHODS

Hens (LSL, Lohmann Selected Leghorn) were obtained from a local farm. Triton X-100 and salmon sperm DNA were from Sigma and EGTA (ethyleneglycol-bis(2-aminoethylether)-N,N'-tetraacetic acid), PMSF, SDS and proteinase K from Merck, Darmstadt. DNAase I, DNA polymerase (Klenow fragment) and DNAase free RNAase were from Boehringer, Mannheim. Labeled deoxyribonucleotide triphosphates were from Du Pont de Nemours, NEN Division. Poly(U)-sepharose was from Pharmacia.

Hybridization probes. β -Globin probe (20) contains roughly 0.7 kb upstream and 1.2 kb coding sequences of the adult β -globin gene. Vitellogenin probe VTG 101 correspond to sequences 5 kb to 11 kb downstream from the transcrip-

tion initiation site (21). Lysozyme probes are subclones from already existing (18) and newly isolated (22) lambda clones from the charon 4A-library of Dodgson et al. (23) or subclones of pFF₂-lys-16 (19).

Preparation of nuclei and digestion with DNase I. Nuclei from oviduct and liver of laying hens were prepared according to Fritton et al. (14) with modifications. Tissues were homogenized in buffer A (0.15 mM spermine, 0.5 mM spermidine, 60 mM KCl, 15 mM NaCl, 2 mM EDTA, 0.5 mM EGTA, 15 mM TrisHCl pH 7.4) supplemented with 0.5 M sucrose and 1 mM PMSF (freshly added from a 0.1 M stock in isopropanol). The pelleted nuclei (2000 x g for 10 min at 4°C) were washed with the same buffer in the presence of 0.5% Triton X-100 until the nuclei were nearly white. In order to remove the detergent, two additional washing steps were done in buffer A supplemented with 0.35 M sucrose and 1 mM PMSF. Pelleted nuclei were frozen in liquid nitrogen and stored at -80°C.

Frozen nuclei were thawed and suspended at a DNA concentration of 200-1000 µg/ml in 0.15 mM spermine, 0.5 mM spermidine, 60 mM KCl, 15 mM NaCl 0.2 mM EDTA, 0.2 mM EGTA, 15 mM Tris HCl pH 7.4, and 1 mM freshly added PMSF and digested with various amounts of DNase I in the range between 15 to 4000 U/ml at 0°C for 10 min. Digestions were started by the addition of 5 mM MgCl₂ and stopped by addition of 10 mM EDTA. Digested nuclei were spun down for 1 min at 2000 x g and washed once or twice in buffer A. DNA from digested nuclei was isolated by proteinase K treatment as described (14).

Restriction nuclease treatments and analyses of DNA fragments by gel electrophoresis, blotting, hybridization with nick-translated probes, and autoradiography was as described (14).

For slot-blot analyses DNA was denatured in 0.3 M NaOH at 65°C for 30 - 60 min. After cooling to room temperature the solution was neutralized by adding an equal volume of 2 M ammonium acetate, pH 7.0 and 5 µg spotted to nitrocellulose filters by using the slot-blot apparatus from Schleicher & Schüll. The filters were baked for 2 h in a vacuum oven at 80°C and then hybridized as described (14).

Isolation of cellular RNA from the oviduct of hens, enrichment of poly(A) containing mRNA and endlabelling. Total cellular RNA was prepared from oviduct immediately after animal sacrifice. About 2 g of tissue was blended 6 x for 30 seconds in 20 ml of 3M lithium chloride, 6M urea, 0.3 M sodium acetate pH 6.0 and kept overnight at 0°C. The precipitate was pelleted for 20 min at 15.000 x g. The pellet was dissolved in 20 ml of 50 mM sodium acetate pH 5.5, 10 mM EDTA, 0.5% SDS saturated with phenol, twice extracted

with phenol, once with phenol-chloroform (1:1) and twice with chloroform-isoamylalcohol (24:1). To the aqueous phase 0.1 volume of a 5M ammonium acetate solution was added and the RNA precipitated with 2.5 volumes of ethanol. RNA was dissolved in 1-2 ml elution buffer (10 mM TrisHCl pH 7.5, 1 mM EDTA, 0.2% SDS) and heated for 5 min at 65°C. The solution was made 0.5M of NaCl and 1-2 ml loaded onto 1 ml poly(U)-sepharose columns. The RNA running with the loading buffer was reloaded twice. Poly(A)-minus-RNA was eluted with 20-30 ml of loading buffer at 20°C. Poly(A)-containing mRNA was eluted with 10 ml of elution buffer at 38°C. Elution was controlled with a UV-monitor and the mRNA immediately precipitated with ethanol, dried and stored at -20°C.

RNA was dephosphorylated with alkaline phosphatase and 5' end-labeled with polynucleotide kinase (24,25). Hybridizations of slot-blots with labeled RNA were done similarly as with nick-translated cloned DNA (14) except that during the posthybridization washing procedures one RNase step was included. Filters were treated with 20 µg/ml RNase for 30 min at 37°C in 2 x SSC.

RESULTS

The lysozyme gene in oviduct nuclei resides within an approximately 24 kb long domain of enhanced DNase I-sensitivity

We pursued the question of how far the enhanced DNase I-sensitive chromatin conformation extends into the 5' and 3' nontranscribed regions of the lysozyme gene. Nuclei from hen oviduct, liver and erythrocytes were digested with increasing amounts of DNase I. DNA from digested nuclei was purified and the length-distribution of the fragments analyzed on neutral 1.5% agarose gels (not shown). Equal amounts of undigested DNA and of digests with DNA mean lengths between 5000 bp and 300 bp were applied to nitrocellulose filters and the relative concentrations of various nucleotide sequences assessed by the slot-blot hybridization assay. As a quantitative measure of DNase I sensitivity we determined the DNase I concentrations that were necessary to degrade 50% of the chromatin fragment in question to non-hybridizable sizes.

Fig. 1A shows that in oviduct nuclei twice as much DNase I was necessary to degrade 50% of the DNA recognized by probes A and J as compared to DNA recognized by probe E (for the location of the probes relative to the gene see Fig. 3). This means that DNA corresponding to probe E, which is within the structural gene, has twice the sensitivity of DNA recognized by probe A, which is about 20 kb in front of the transcription start site, or sequences recog-

Figure 1. Selected sequences in and around the lysozyme gene as well as from other reference genes were assayed for their sensitivity to DNase I digestion. A: 5 μ g of DNA from oviduct nuclei digested with increasing amounts of DNase I (0, 125, 250, 500, 1000 and 2000 U/ml) were spotted onto a nitrocellulose filter. The filter was first hybridized with 32 P-labeled probe A and subjected to radioautography. After removal of the radioactivity the same filter was hybridized with probe E. Another filter prepared exactly in the same way was used for hybridization with probe J. B: The same three regions of the lysozyme gene were assayed for their relative sensitivity to DNase I in nuclei from erythrocytes. The same filter was used for subsequent hybridizations with all three probes. C: The sensitivity of the β -globin and vitellogenin gene was measured in liver nuclei. For length and location of hybridization probes in relation to the lysozyme gene see Fig. 3. In the upper part, scans are presented from autoradiograms that were exposed one third as long as those shown in the middle part. The peak areas were determined and plotted as function of the DNase I concentration (lower part). DNase I concentrations needed for digestion of 50% of the fragment in question to unhybridizable sizes were determined by interpolation and are indicated by the vertical arrows.

nized by probe J, which is about 20 kb behind the poly A site. Using the same three probes, no difference in sensitivity was observed in erythrocytes (Fig. 1B) that do not express the lysozyme gene. The same is true for liver (not shown). This confirms that the lysozyme gene is in a sensitive domain in the tissue that expresses the gene. As a further control we probed DNA from digested liver nuclei with a vitellogenin and β -globin probe. The vitellogenin gene active in liver shows a clearly higher sensitivity to DNase I than the inactive β -globin gene (Fig. 1C).

In order to determine the borders of the domain, we assessed the relative DNase I-sensitivity of sequences flanking the lysozyme gene. The hybridization probes covered about 50 kb of DNA around the gene. Fig. 2 shows that DNA sequences corresponding to probe B are only slightly more sensitive than

Figure 2. DNase I sensitivity of selected sequences in and around the lysozyme gene in oviduct nuclei. 5 μ g of DNA from oviduct nuclei digested with increasing amounts of DNase I (0, 125, 250, 500, 1000 and 2000 U/ml) were spotted onto 8 separate stripes of nitrocellulose. Each filter was hybridized subsequently with two or three probes (capital letters). For lengths and location of hybridization probes in relation to the lysozyme gene see Fig. 3. Autoradiograms were scanned and the peak areas plotted as function of DNase I concentration. DNase I concentrations needed for digestion of 50% of the fragments are indicated by vertical arrows and used for estimation of the relative sensitivity of various regions around the lysozyme gene. The results are summarized in Fig. 3.

sequences corresponding to probe A. On the other hand, the C-region is twice as sensitive as the A-region. These findings indicate that the 5' border of the domain is somewhere near B (Fig. 3). On the 3' side the DNA recognized by probe G shows high sensitivity compared to the I- or K-region, while the DNA corresponding to H is possibly in between. This places the 3' border close to the region recognized by probe H. Outside of the domain the sensitivities are comparable since the A-region is as resistant as J or K. The fragment combinations in the different panels were chosen such that one fragment was from the putatively sensitive region while the other one, or two other fragments, were from the resistant region. For reasons of accuracy, we used the same filter consecutively for two or three hybridizations with the probes to be compared directly. The results shown in Fig. 2 and Fig. 1A were from a single set of experiments. However, the summary of our results presented in Fig. 3 is based on four independent and two semi-independent sets of experiments. We consider results obtained with different preparations of nuclei to be inde-

Figure 3. The DNase I sensitive domain of the lysozyme gene was determined from the results of Figs. 1 and 2, and from further five sets of experiments (not shown). Mean values (•) and total deviations (vertical bars) are indicated. The gene is drawn as a box, exons depicted in black and introns in white. The horizontal arrow indicates start and direction of transcription. Vertical arrows mark the position of DNase I-hypersensitive sites revealed by the experiments of Figs. 6 and 7, and from previous data (14,17). Lengths and positions of hybridization probes are shown as bars labeled by capital letters. The open boxes in the third line indicate the position of repetitive sequences. A scale in kb and maps of restriction nuclease cleavage sites pertinent to this work are shown in the lower part. E=EcoRI; H=HindIII; B=BamHI.

pendent whereas in semi-independent experiments, a certain batch of nuclei was used for a number of digestions.

In conclusion, the lysozyme gene in oviduct nuclei is contained within an approximately 24 kb domain of enhanced DNase I sensitivity. The 5' boundary of this domain is rather broad. It spans a region 6-7 kb in length, along which the sensitivity decreases slowly. The 3' boundary, on the other hand, seems more defined.

Experiments based on the "first cut" approach

In the previous section, DNase I-sensitivity of chromatin was monitored by following the course of digestion to yield fragments too small to be retained on filters. We refer to this as the "last cut" approach. An alternative possibility is to follow the rate of disappearance of specific restriction bands by gel electrophoresis and Southern blot hybridization. Since the first double strand cut by DNase I eliminates the restriction band in question, we call this procedure the "first cut" approach.

Fig. 4a demonstrates that restriction bands within the sensitive domain are more susceptible to DNase I than fragments outside of the domain, in good agreement with the previous approach. In particular, the two EcoRI fragments E4-E5 and E5-E6, which cover the whole gene and extend into flanking sequences, disappear more rapidly than fragment E(-1)-E1, that overlaps with the 5' boundary region. The most resistant fragment in this series is E11-E12 that is downstream of the sensitive domain (for the location of restriction fragments relative to the gene see Fig. 3). A similar result was obtained using Hind III derived fragments (Fig. 4b). H9-H10 from the structural gene region is the most sensitive fragment; H12-H13 which is located outside of the domain is the most resistant one, whereas H1-H2a that originates from the 5' boundary region is of intermediate sensitivity. We show (Fig. 4c) that the same three fragments are similarly resistant in erythrocytes in which the lysozyme gene is not in a sensitive domain.

We demonstrate that sequences outside of the sensitive domain (E8-E9 and E11-E12) are as resistant in oviduct as the inactive β -globin reference gene (Fig. 4d). On the other hand, when controls were performed with nuclei from erythrocytes in which β -globin is located in an open domain, it showed a higher sensitivity than the lysozyme gene (Fig. 4e). Fig. 4f confirms again that a fragment residing within the domain (B4-B5) is more sensitive than a fragment situated outside (B6-B7).

In qualitative terms, both the "first cut" and "last cut" approach gave consistent results. In order to obtain a quantitative estimate, we scanned the

Figure 4. (A) Relative DNase I sensitivity of specific restriction fragments from the lysozyme and β -globin gene regions. Nuclei from oviduct (a,b,d,f) and erythrocytes (c,e) were treated with increasing DNase I concentrations (0, 15, 30, 60, 125, 250, 500 and 1000 U/ml). DNA was purified by deproteinization and restricted with either EcoRI, HindIII or BamHI. After 1% agarose gel electrophoresis DNA was blotted to nitrocellulose filters and hybridized with ^{32}P -labeled nick translated plasmids. Lengths of fragments is given on the left in kb; designation of the fragments is given on the right. For the location of individual restriction fragments see Fig. 3. (B) Autoradiograms shown in (A) were scanned and the peak areas plotted in a double-logarithmic plot as function of the DNase I-concentration (given in multiples of initial concentration). Fragments that lay within the sensitive domain or in the transition region are labeled by open symbols, those from the resistant parts by filled symbols.

autoradiograms and plotted the peak areas in a double-logarithmic plot, as a function of DNase I concentration (Fig.4B). The values fall well on straight lines with different slopes. The tangents of the angle is a quantitative measure of the sensitivity of a fragment. However, this kind of sensitivity depends on various factors, the most important one being the fragment size. The presence of DNase I-hypersensitive sites also influences the sensitivity as measured by this particular approach. Once correction for size is made (not shown), the fragments in the sensitive domain display 1.5 to 4.8 times higher sensitivities than those outside of the domain. Those carrying hypersensitive sites are the most sensitive ones.

Cloning work and location of repetitive DNA

We have performed additional cloning and restriction mappings of far upstream and downstream sequences. This became necessary as the regions known did not extend much beyond the sensitive domain. Most details of this cloning work, including the location of repetitive sequences, is presented elsewhere (22). Results of restriction mapping experiments pertinent to this work are shown in Figs. 3 and 6. New repetitive DNA sequences were detected in addition to those previously known approximately 20 kb downstream of the cap site (18,19,22). As shown in Fig. 3 no repetitive DNA could be detected further than 6 kb upstream of the transcription start site and none are present close to the 5' boundary of the domain. Apparently, repetitive DNA sequences are not necessary elements at the boundaries of the lysozyme domain as was suggested previously for other genes (8,9).

Analysis of transcriptionally active regions

Fig. 5 shows that in oviduct cells only the lysozyme gene is actively transcribed within the region analyzed. Labeled poly(A)-containing RNA hybridizes only with plasmid DNA sharing DNA sequences with the gene itself (probes d,E,f). Our experiments give no indication for polymerase III transcripts of repetitive DNA in this region, in agreement with the findings of others (19). Although we used fractions enriched in poly(A⁺) RNA, we still would expect hybridization signals for transcripts without a poly(A) tail (polymerase III products), since RNA separations have never been complete in our hands. Apparently, polymerase III transcripts of repetitive DNA from the lysozyme gene region do not play an important role in the in vivo control of lysozyme gene expression in oviduct cells.

Figure 5. Only the lysozyme gene itself is transcribed in oviduct cells within the sensitive domain and their flanking sequences. 5 µg of plasmid DNA carrying hen sequences as indicated by the bars were spotted onto a nitrocellulose filter strip and hybridized with ³²P-labeled poly(A) containing RNA from the oviduct of hens. DNA clones labeled with upper case letters were the same as used for determination of the borders of the domain (Fig. 3). The decreasing intensity of the hybridization signal in 3' to 5' direction indicate partial degradation of the RNA probe used for hybridization.

DNase I-hypersensitive sites are clustered within the sensitive chromatin domain

In a previous study we characterized eight nuclease-hypersensitive chromatin sites around the lysozyme gene in oviduct, five upstream, and three downstream of the transcription unit (14). In contrast, only one such site was revealed in transcriptionally inactive erythrocytes. This and other findings indicated a functional relationship of most of the nuclease-hypersensitive sites with active states of the lysozyme gene (17). In this work we obtained further indication for a close relationship between hypersensitive sites and functional aspects of the lysozyme gene by the fact that all (but one) of the previously mapped sites are contained within the sensitive domain (Fig. 3).

In order to confirm the clustered appearance of DNase I-hypersensitive sites around the lysozyme gene, we examined regions even further upstream and downstream than in previous investigations (14,17). The experiments described in the following, together with the previous mapping experiments, cover a contiguous stretch of 60 kb around the lysozyme gene. For mapping of DNase I-hypersensitive sites we utilized the indirect end-labeling technique of Wu (27). No DNase I-hypersensitive site could be detected in the region between -7.9 kb and -26 kb with respect to the promoter (not shown). Several weak bands which were visible in some of our autoradiograms were due to preferential cutting of DNase I at specific DNA sequences, rather than to true hypersensitive chromatin sites. When we searched for DNAase I-hypersensitive sites downstream of the promoter one new site was revealed (Fig. 6B-D) which, however, is clearly outside of the domain of general DNase I sensitivity. Its distance from the promoter is +22.9 kb. An updated map of DNase I-hypersensitive sites is shown in Fig. 3. These sites are clearly clustered around the lysozyme gene in a region not longer than 7.9 kb upstream of the promoter and 4 kb downstream of the poly A site.

A site referred to as HS7 in our earlier paper (14) has been the least prominent one and appeared only with DNAase I, but not with the endogenous nuclease from oviduct nuclei. Since naked DNA shows inherent cutting preference with DNAase I at this site, we no longer consider this to be a true DNAase I-hypersensitive chromatin site.

DISCUSSION

The present study is part of our attempts towards an understanding of the structural basis of controlled expression of the lysozyme gene. Controlled expression seems to require that a gene exist in a more open chromatin confor-

Figure 6. Mapping by indirect end-labeling of DNAase I-hypersensitive sites in the far downstream region (+7.8 kb to +34 kb) of the lysozyme gene in oviduct of laying hens. Digestion of nuclei with two different DNAase I-concentrations (lanes a,b) or different incubation times with endogenous nuclease (lanes c,d) are shown; m, chain length markers, the lengths given in kb. The regions investigated are indicated by thick horizontal lines. The hybridization probes used are shown as bars below the restriction maps. E, Eco RI; B, Bam HI; X, Xba I. The box represents the lysozyme gene, the four exons are depicted in black, and three introns in white. The direction of transcription is shown by the horizontal arrow. Vertical arrows are DNAase I-hypersensitive sites in oviduct chromatin named according to their approximate distance in kb from the transcription initiation site (-, upstream; +, downstream). 1% agarose gels were used.

mation as reflected by enhanced sensitivity to DNase I (1-4). This open structure is not confined to the transcribed region itself but extends many kb upstream and downstream of the gene to give large DNase I-sensitive domains with more or less defined boundaries (8-10). This enhanced sensitivity manifests itself either as a rapid conversion of the entire sequence into short non-hybridizable fragments upon extensive DNase I digestion ("last cut approach"), or as a rapid disappearance of specific restriction bands under mild digestion conditions ("first cut approach").

Utilizing both the "first cut" and the "last cut" procedures we obtained consistent results concerning the extent of DNase I sensitivity of the active lysozyme gene and the location of the boundaries of the sensitive lysozyme domain. The 3' boundary was determined with an uncertainty of 4 kb. A more accurate determination was precluded by the presence of repetitive DNA inbetween

our probes G and H (Fig. 3).

The transition from the sensitive to a resistant structure at the 5' boundary occurs in a graded fashion. A similar situation has been previously observed by O'Malley and coworkers in the ovalbumin (8) and GAPDH (9) domain. The molecular basis of both, the DNase I sensitive chromatin conformation and the gradual fall of this sensitivity at the 5' boundary of active domains, are not fully understood. Major features of DNase I-sensitivity have been attributed to an altered core particle structure. HMG proteins (28), changed histone composition (29), histone modification (e.g. 30), among others, are believed to be responsible for inducing the sensitive nucleosome conformation. A gradual change in the ratio of modified and unmodified nucleosomes along the chromatin fiber, possibly as a consequence of differential torsional stress (e.g.11) may explain the gradual change in DNase I sensitivity. Enhanced DNase I sensitivity has also been attributed to an unwinding of the solenoid structure (10,31) rather than to nucleosome core modification. A gradual change in DNase I sensitivity can be explained also on this basis, provided that spiralization of the chromatin filament to yield a solenoid starts at various points along the DNA in a population of cells. Other explanations for a broad border of sensitive domains have been given previously (8,9).

One aspect of this study pertains to the distribution of DNase I-hypersensitive sites along chromosomal regions. In the case of the lysozyme gene these sites are situated in a cluster upstream and downstream of the transcription unit, and with a single exception, all within the sensitive domain. This new observation points to their connection with functional aspects of the lysozyme gene. Such a clustered arrangement of hypersensitive sites has not been demonstrated for other genes so far.

The presence of DNase I-hypersensitive sites thousands of base pairs away of the gene, but within the sensitive domain, points to the possibility that the lysozyme gene is controlled also from remote sites. This view was strongly supported by recent observations that the tissue and expression specific hypersensitive site 6.1 kb in front of the gene is a target for the binding of the TGGCA protein or nuclear factor 1 (NFI) (15,16,32), a factor also involved in the regulation of an oncogene (33) and viral genes (16,26). DNA sequences corresponding to site HS -6.1 show enhancer activity (34).

Our present work was set out to define the chromosomal region structurally and functionally related to the lysozyme gene. Our knowledge of the borders of the gene-domain and the precise location of DNase I-

hypersensitive sites within the domain provided structural data important for further studies on functional aspects of lysozyme gene regulation.

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NOTE ADDED

After having finished this manuscript two papers appeared showing that actively transcribed human globin genes lie within larger domains of moderate sensitivity encompassing the entire gene clusters. Forrester et al. (1986). *Proc. Natl. Acad. Sci. USA* 83,1359 and Yagi et al. (1986). *Mol. Cell. Biol.* 6,1108.