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Chromatin Structure of the β-Globin Gene Family in Murine Erythroleukemia Cells†

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ABSTRACT: We have analyzed the chromatin structure of the β-major globin gene and other related β-globin genes in induced and uninduced murine erythroleukemia (MEL) cell nuclei. Nuclei were digested with either DNase I or micrococcal nuclease, and the purified DNA was hybridized to a set of cloned genomic DNA fragments covering the β-globin gene region. This region consisted of two distinct domains as characterized by sensitivity to DNase I digestion. One domain was relatively sensitive and contained the potentially active or actively transcribed β-major and β-minor globin genes. The other, relatively insensitive domain contained the nontranscribed embryonic and β-globin homologous genes. The sensitivity of these domains was not altered during erythroid differentiation. In nonerythroid cells, the entire globin gene family, including the adult and embryonic globin genes, was contained in a single relatively resistant domain. Micrococcal nuclease (MNase) also defined two general domains of nuclease sensitivity that coincided with those of DNase I. However, the relatively sensitive MNase domain containing the β-major and β-minor genes became more sensitive upon chemically stimulated erythroid differentiation. A detailed examination of the β-major globin gene revealed that the actual coding region became increasingly sensitive to micrococcal nuclease after differentiation while the 5′-flanking DNA did not. Thus, micrococcal nuclease was able to accurately define the primary transcription unit of the β-major gene.

Recent studies have established that transcriptionally active genes are in chromatin structures with a conformation different from that of inactive genes. Genes that are expressed or have the potential to be expressed exhibit a preferential susceptibility to cleavage by nucleases. Two well-characterized examples are the globin genes in chicken erythrocytes (Weintraub & Groudine, 1976; Stalder et al., 1980a) and the ovalbumin gene in the hen oviduct (Garel & Axel, 1976; Bloom & Anderson, 1979; Bellard et al., 1980, Lawson et al., 1980; Anderson et al., 1983). This enhanced sensitivity is characterized either as a reduction of the sequence under study into small nonhybridizable fragments following extensive digestion or as site-specific cleavages introduced into unique hypersensitive sites near the gene under mild digestion conditions (Stalder et al., 1980a,b; Weintraub et al., 1981; Anderson et al., 1983). In these as in other cases, nuclease sensitivity or hypersensitivity correlates with the differentiated state of the cell rather than the actual transcription of the genes. Since there have been no reports of an expressed gene being in a nuclease-resistant state, the acquisition of nuclease sensitivity appears to be prerequisite to eukaryotic gene expression.

It has been recently shown in several specific systems that active genes reside within large DNase I sensitive domains that include not only the coding regions but also large regions of nontranscribed sequences as well. The ovalbumin gene and neighboring X and Y genes in hen oviduct nuclei are packaged into a DNase I sensitive domain approximately 100 kb in length (Lawson et al., 1982). These three genes are transcribed at different rates (Colbert et al., 1980; Lemur et al., 1981) and are equally sensitive to DNase I (Garel et al., 1977) and remain so during their transcriptional inactivation upon steroid hormone withdrawal (Lawson et al., 1982). The chicken α- and β-globin genes are also packaged into large DNase I sensitive domains in chicken erythroid cells (Stalder et al., 1980a,b) and remain sensitive following transcriptional termination in the quiescent red blood cell nucleus.

The extent of large DNase I sensitive domains containing active genes has been mapped in several cases. One such domain has been shown to extend as little as 3–4 nucleosomal DNA lengths beyond the 5′-end and 2–15 nucleosomal DNA lengths beyond the 3′-end of integrated adenovirus genes in transformed hamster cells (Flint & Weintraub, 1977). On the other hand, Bellard et al. (1980) have shown that DNase I sensitivity extends at least 30 nucleosomal DNA lengths beyond the 3′-side of the ovalbumin coding region. Recently, Lawson et al. (1982) mapped the DNase I sensitive domain containing the ovalbumin, X, and Y genes in hen oviducts and found this region to extend 30 kb to the 5′-side of the X gene and greater than 20 kb to the 3′-side of the ovalbumin gene.

Studies using micrococcal nuclease (MNase) have shown that the ovalbumin gene in hen oviduct nuclei is preferentially

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digested compared to inactive genes or bulk DNA (Bellard et al., 1978; Bloom & Anderson, 1978, 1979). In contrast to DNase I, MNase recognizes some feature of albumin gene chromatin that changes during the hormone-stimulated transcription of this gene (Bloom & Anderson, 1979). Also, globin gene chromatin becomes insensitive to MNase during gene inactivation, which accompanies erythroid cell maturation (Bloom & Anderson, 1979). Such observations support the concept that large DNase I sensitive domains represent a primary organization of active chromatin within which reside smaller domains of chromatin structure detectable by MNase and which more closely reflect the transcription process.

Miller et al. (1978) have demonstrated that the adult β-globin genes reside in a chromatin structure equally sensitive to DNase I in both induced and uninduced murine erythroleukemia cells. However, little is known about the chromatin structure of the β-globin gene family in these cells. Using cloned DNA fragments as hybridization probes, we have investigated the extent of DNase I and MNase sensitivity in the β-globin gene complex in murine erythroleukemia (MEL) cells. The mouse β-globin gene family has been extensively characterized (Jahn et al., 1980) and is of interest because the adult globin genes, β-major and β-minor, are coordinately expressed during chemically stimulated erythroid differentiation in MEL cells [for a review, see Marks & Rifkind (1978)]. Thus, these cells serve as a model system in which to study gene regulation and chromatin structure during cellular differentiation.

Experimental Procedures

Cell Culture. Stock cultures of DS19, a subclone of Friend erythroleukemia cell line 745 derived from DBA/2J mice (Ohta et al., 1976) were grown in Eagles minimal essential media (MEM) supplemented with 100 units of penicillin/mL, 100 μg of streptomycin/mL, and 15% fetal calf serum. Cells were induced by the addition of HMBA[1] to a final concentration of 4 mM. Cell viability was determined by Trypan blue dye exclusion. The number of cells containing hemoglobin was assayed by benzidine staining (Orkin et al., 1975). Induced cultures, after 72 h, routinely contained more than 95% benzidine-positive cells. The myeloma cell line MOPC104E (a gift from Dr. S. McMillan) was grown as an ascites tumor in pristane-primed Balb/C mice.

Isolation and Digestion of Nuclei. Cells were washed 3 times in RSB (10 mM Tris-HCl, pH 7.6, 10 mM NaCl, and 5 mM $MgCl_2$) and lysed in a Dounce homogenizer. Nuclei were pelleted, washed twice with RSB, and resuspended at 40 A_{260} units/mL. Nuclei either were digested in RSB with 20 units of DNase I (Boeringer-Mannheim) per mL or were washed and resuspended in 1 mM Tris, 25 mM KCl, 0.9 mM $MgCl_2$, 0.9 mM $CaCl_2$, and 0.14 mM spermidine, pH 7.6 (Sanders, 1978), at 40 A_{200} units/mL and digested with 50 units of micrococcal nuclease (Worthington) per mL. All digestions were performed at 37 °C.

Filter Hybridizations. DNA from either micrococcal nuclease or DNase I digested chromatin samples was prepared by treatment with 0.2% $NaDodSO_4$ and 100 μg of proteinase K/mL at 37 °C for 2 h. The DNA was further purified by extraction with phenol and chloroform followed by precipitation in ethanol. DNA was resuspended in 10 mM Tris-HCl, pH 7.6–1 mM EDTA, and 15 μg of DNA from each sample was bound to nitrocellulose membranes by the method of Robinson et al. (1982). Restriction fragments were obtained with the appropriate restriction endonucleases and isolated by agarose gel electrophoresis (Roop et al., 1980). The cDNA clone for the εY3 gene was originally described by Fantoni et al. (1980), and the cDNA clone for the immunoglobulin heavy-chain gene was isolated by Davis et al. (1980). Radioactive probes were prepared by nick translation (Maniatas et al., 1975) and routinely had a specific activity of 5×10^7 cpm/μg. Probe DNA was in excess compared to filter-bound DNA, and hybridizations were performed in 6× SSC, 1× Denhardt's solution (Denhardt, 1966), and 0.5% sodium pyrophosphate at 65 °C for 48 h. Filters were extensively washed at 65 °C in 6× SSS, 3× SSC, and finally 1× SSC.

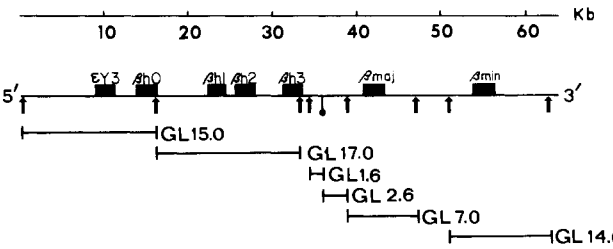

FIGURE 1: Map of the β-globin genes in MEL cells. Cloned restriction fragments are designated GL followed by their lengths in kilobase pairs. The map indicates the positions of pertinent *Eco*RI (↑) and *Bam*HI (●) restriction sites.

Analysis of Nuclease Digestions. Following the hybridization of radioactive probes to nuclease-digested DNAs under conditions of probe-sequence excess, the amount of filter-bound radioactivity was determined by scintillation counting. The fraction of filter-bound cpm for nuclease-digested DNA divided by the cpm bound to undigested total mouse DNA was calculated. The log of this fraction was taken, resulting in a negative number, a hence, −log hybridization was plotted against time. For times of digestion up to 30 min, such plots gave straight lines from which the slope was calculated and the relative rate of digestion was determined. Each experiment was performed at least 2 times. Standard deviations were calculated for each set of relative rates, and the average rates are reported.

Transcription in Isolated Nuclei and Dot Hybridization. MEL cell nuclei were prepared as described above. The standard labeling assay described by Hofer & Darnell (1981) used 125 μCi of [α-^{32}P]UTP (3000 Ci/mmol, New England Nuclear) per 10^8 nuclei. Incorporation at 37 °C was in the linear range up to 20 min, and preparations were labeled for 10 min. Cloned DNA sequences for the β-major/β-minor genes (Konkel et al., 1978) and the εY3 gene (Fantoni et al., 1979) were bound to nitrocellulose following denaturation in 0.2 N NaOH and neutralization in 2 mM HEPES. Approximately 10 μg of each DNA was bound per dot. Hybridizations were performed as described above. Following hybridization, the samples were treated with 2.5 μg/mL RNase A and 5 μg/mL RNase Ti at 37 °C for 40 min. The RNase-resistant hybrids were quantitated by scintillation counting.

Results

Sensitivity of the β-Globin Gene Family to DNase I. A map of the β-globin gene family, indicating the relative positions of the cloned *Eco*RI restriction fragments used as hybridization probes in this study, is presented in Figure 1. Globin gene DNA fragments were designated as GL followed by the approximate length of each fragment in kilobase pairs. Fragments GL15, GL17, and GL14 were originally described by

[1] Abbreviations: HMBA, hexamethylenebis(acetamide); SSC, standard saline citrate; Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Table I: Rate of Digestion for Specific Regions of Globin Gene Chromatin[a]

	relative rates of nuclease digestion			
	DNase I		MNase	
fragment	HMBA−	HMBA+	HMBA−	HMBA+
GL15.0	0.012 ± 0.001[b]	0.011 ± 0.001	0.0066 ± 0.0002	0.0065 ± 0.0003
GL17.0	0.011 ± 0.002	0.012 ± 0.001	0.0064 ± 0.0004	0.0066 ± 0.0005
GL1.6	0.012 ± 0.002	0.012 ± 0.003	0.0081 ± 0.0003	0.0085 ± 0.0002
GL2.6	0.028 ± 0.002	0.027 ± 0.001	0.0124 ± 0.001	0.0165 ± 0.001
GL7.0	0.029 ± 0.001	0.030 ± 0.003	0.0125 ± 0.001	0.0320 ± 0.003
GL14.0	0.030 ± 0.002	0.028 ± 0.004	0.0125 ± 0.001	0.020 ± 0.004
Cμ12	0.012 ± 0.02	0.013	0.0065 ± 0.001	0.0064 ± 0.001

[a] The rates of digestion were calculated from the slopes of lines shown in Figures 1 and 2. The standard deviations were calculated from values obtained from at least two experiments. [b] Standard deviation.

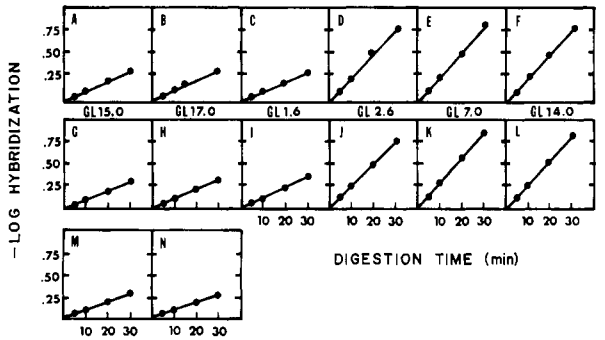

FIGURE 2: DNase I sensitivity of specific sequences within the β-globin gene family. DNase I digestion and hybridizations were performed as described under Experimental Procedures. (A–F) Kinetics of hybridization for the specific cloned sequences following DNase I digestion of uninduced MEL cell nuclei. (G–L) Kinetics of hybridization for the same sequences following digestion of induced MEL cell nuclei. DNase I digested uninduced DNA (M) and induced DNA (N) were also hybridized with the immunoglobulin gene probe Cμ12.

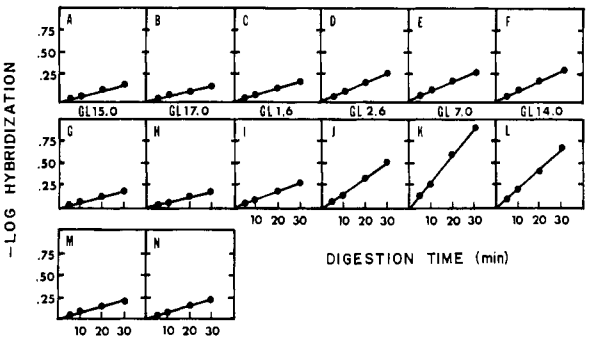

FIGURE 3: MNase sensitivity of sequences within the β-globin gene family. MNase digestion and hybridization were performed as described under Experimental Procedures. (A–F) Kinetics of hybridization for the specific cloned sequences following DNase I digestion of uninduced MEL cell nuclei. (G–L) Kinetics of hybridization for the same sequences following digestion of induced MEL cell nuclei. DNase I digested uninduced DNA (M) and induced DNA (N) hybridized with the immunoglobulin gene probe Cμ12.

Jahn et al. (1980) and correspond to CE18, CE17, and CE14 in their nomenclature, respectively. GL7.0 corresponds to the cloned 7-kb *Eco*RI restriction fragment isolated from λgtWESβG2 (Konkel et al., 1978). The 1.6- and 2.6-kb fragments were generated by *Bam*HI restriction of the 4.2-kb *Eco*RI fragment carried by a clone designated CA4 (Dr. C. A. Hutchinson, III, personal communication). These fragments were used to detect their respective sequences in nuclease-digested MEL cell DNA.

In order to define DNase I sensitive domains within the β-globin gene family, we have examined the DNase I sensitivity of globin gene chromatin in induced or uninduced MEL cells. MEL cells were induced to differentiate by incubation with 4 mM HMBA for 72 h at which time the population contained more than 98% benzidine-positive cells. Uninduced MEL cell populations contained less than 2% benzidine-positive cells. Purified nuclei were digested with DNase I for various lengths of time, and the relative rate of digestion was calculated for each of the specific regions in the globin gene complex. The extent of digestion was such that approximately 20% of the DNA was rendered acid soluble by 30 min. Figure 2A–F shows the hybridization results from uninduced cells and Figure 2G–L the results from HMBA-induced cells. DNase I was able to detect two distinct domains in both induced and uninduced MEL cells. The DNA sequences detected by restriction fragments GL15.0, GL17.0, and GL1.6 were relatively resistant to digestion compared to DNA that was hybridized to fragments GL2.6, GL7.0, and GL14.0. The slopes from the graphs shown in Figure 2 were calculated and the relative rates of digestion reported in Table I. The sensitivities of the embryonic and β-globin homologous (βh) genes were approximately the same as the sensitivity of the immunoglobulin heavy-chain gene, Cμ12 (Davis et al., 1980), which is also not transcribed in MEL cells.

The two adult globin genes, β-major and β-minor, which are expressed during differentiation, were found to reside in a relatively sensitive domain of at least 25 kb in length. The domain containing the adult globin genes was digested by DNase I at a rate at least twice as fast as the domain containing the βh and embryonic genes; the relative sensitivity of this domain did not change during HMBA-stimulated erythroid differentiation (Table I). The transition region between these two distinct domains was very precise and was located approximately 3860 bp upstream from the β-major gene cap site and 2500 bp to the 3′-side of the βh3 globin gene. This would place the transition area approximately 18–20 nucleosome lengths away from the 5′-end of the β-major gene.

Micrococcal Nuclease Sensitivity of the β-Globin Gene Family. In order to investigate the chromatin structure of the β-globin gene family with MNase, nuclei were digested as described under Experimental Procedures, and the purified DNA was hybridized with the various cloned genomic DNA fragments. The analysis of DNA fragments within the β-globin gene complex following MNase digestion of uninduced nuclei is shown in Figure 3A–F. Hybridization results from uninduced cells are shown in Figure 3G–L. The rates of digestion are summarized in Table I. In uninduced MEL cells, there appear to be two domains of MNase sensitivity with a transition in the region between the βh3 and β-major genes. The transition between these domains was less distinct compared to the boundary detected by DNase I. The β-major and β-minor genes were located in a chromatin structure approximately 2 times more sensitive to MNase than the region containing the βh and embryonic genes. When HMBA-in-

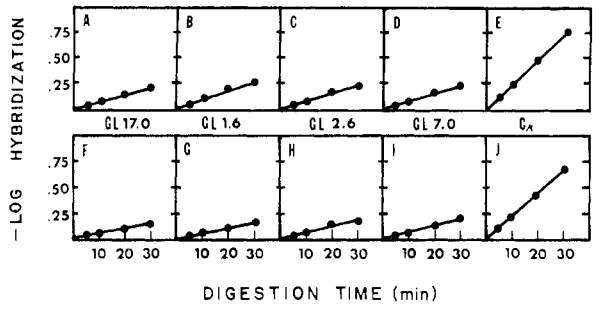

FIGURE 4: DNase I and MNase sensitivity of globin genes in nonerythroid cell nuclei. Labeled DNA fragments GL17.0, GL1.6, GL2.6, and GL7.0 were hybridized to either DNase I (A–D) digested or MNase- (F–I) digested DNA from MOPC104E cell nuclei. As a control, the immunoglobulin cDNA probe, $C\mu 12$, was hybridized to DNase I (E) digested and MNase- (J) digested DNA from the same nuclei.

duced cells were examined, the rates of MNase digestion in the regions probed by GL15.0, GL17.0, and GL1.6 were very similar to the rates obtained from uninduced cells. However, the rates of digestion for the region containing the β-major gene (GL7.0) increased approximately 2.5 times, and the digestion rate for the region containing the β-minor gene (GL14.0) nearly doubled. The data of Figures 2 and 3 show that although the DNase I sensitivity of the active and potentially active sequences was the same, the MNase sensitivity dramatically increased during transcription. These data corroborate a recent report by Anderson et al. (1983) with regard to the MNase sensitivity within the ovalbumin gene family.

DNase I and Micrococcal Nuclease Sensitivity of the Globin Genes in Nonerythroid Cells. In order to determine if the nuclease-sensitive domains defined in MEL cell line DS19 were specific for erythroid cells, the DNase I and MNase sensitivities of the nontranscribed β-globin gene family were examined in the IgM-producing myeloma cell line MOPC104E. Nuclei were prepared and digested as described under Experimental Procedures, and the digested DNA was hybridized with the various DNA fragments shown in Figure 1. As seen in Figure 4, the globin gene family is apparently packaged into a single chromatin domain of at least 30 kb in length as detected by DNase I. The rates of digestion for each of the DNA sequences assayed in this region were substantially slower than that of the actively transcribed IgM sequence within the same cells. The rates of MNase digestion also defined a single domain containing the globin gene complex that was less sensitive than the IgM gene sequence. Although the rates of DNase I and MNase digestion of the β-globin family sequences in MOPC104E cells did not exactly match the rates obtained for the equivalent sequences in MEL cells, the implication of these data is that in nonerythroid cells the globin gene region resides in a single, relatively nuclease-resistant chromatin structure.

Micrococcal Nuclease Sensitivity of the β-Major Gene and Flanking DNA Sequences. From the data presented above, it is clear that the β-major gene lies within a DNase I sensitive domain of at least 25 kb in length in erythroid cells. Within this large domain, the β-major gene resides in a chromatin structure that exhibits an increased MNase sensitivity during erythroid differentiation. In order to further understand the basis of differential MNase sensitivity, we have mapped the MNase sensitivity of the β-major gene and flanking DNA sequences in HMBA-induced and uninduced MEL cells using the cloned DNA sequences described in Figure 5 as hybridization probes.

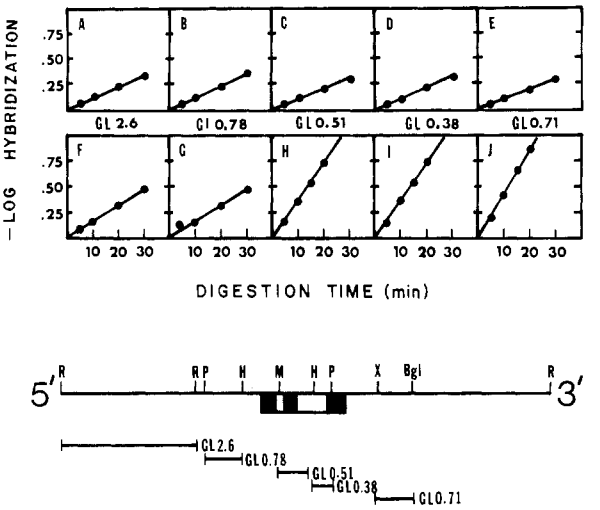

FIGURE 5: MNase sensitivity of specific sequences near the β-major globin gene. Digestions were performed as described under Experimental Procedures. Labeled sequences GL2.6, GL0.78, GL0.51, GL0.38, and GL0.71 were hybridized to MNase-digested DNA from uninduced (A–E) or induced (F–J) MEL cell nuclei. The partial map of the β-major gene and surrounding sequences shows the position of the DNA fragments used as hybridization probes and appropriate restriction sites (Konkel et al., 1978) designated as (R) *Eco*RI, (P) *Pst*I, (H) *Hin*dIII, (M) *Mbo*I, (X) *Xba*I, and (Bgl) *Bgl*II.

Table II: Rates of MNase Digestion for DNA Regions near the β-Major Globin Gene[a]

fragment	relative rates of MNase digestion	
	HMBA−	HMBA+
GL2.6	0.0124	0.0160
GL0.78	0.0124	0.0150
GL0.51	0.0125	0.0420
GL0.38	0.0123	0.0425
GL0.71	0.0126	0.0435

[a] Rates of digestion were determined by calculating the slope of linear plots of -log hybridization vs. time of digestion shown in Figure 5.

Nuclei from induced and uninduced cells were purified and digested as previously described. Figure 5A–E shows the hybridization of various probes surrounding the β-major gene to digested DNA from uninduced nuclei, and Figure 5F–J shows the results from induced cells. The rates of digestion summarized in Table II show that in the uninduced cell flanking DNA and coding-region DNAs were digested at similar rates. The rates of digestion for these gene regions are nearly the same as those detected with the GL7.0 probe in uninduced cells as shown in Figure 3, suggesting that the entire region lies within a domain of uniform chromatin structure. In induced cells where the β-major gene is transcribed, significant changes in the MNase sensitivity of the β-major coding region occur. Hybridization with DNA probes GL0.51, GL0.38, and GL0.71 defined a region of nuclease sensitivity approximately 4 times greater than the 5′-flanking DNA (Table II). In addition, when a β-major cDNA sequence was used as the hybridization probe, its rate of digestion was similar to that obtained in these experiments for GL0.38 and GL0.51 (Yu et al., 1983). The rates of digestion for the coding and 3′-flanking DNA sequences were greater than the rate obtained for the entire GL7.0 fragment (compare Tables I and II), suggesting that within this 7-kb fragment exist small subdomains of altered MNase sensitivity.

Transcription of the Embryonic and Adult β-Globin Genes. The expression of individual genes within the β-globin gene

Table III: Transcriptional Stimulation of the Embryonic and Adult β-Globin Genes[a]

filter-bound DNA	hybridized cpm/10^6 input cpm		hybridized cpm above background		rate stimulation
	uninduced	induced	uninduced	induced	
pBR322	49 ± 7	51 ± 2			
ϵY3	49 ± 4	50 ± 10	0	0	
β-globin	63 ± 6	110 ± 4	14	60	4.3

[a] RNA labeled in isolated nuclei from either uninduced or HMBA-induced cells was hybridized to filter-bound DNA, and the RNase-resistant hybrids were analyzed. Cpm values from at least two experiments are reported with their standard deviation.

family in MEL cells depends upon the cell line and the chemical inducer used. The MEL cell line in this study, DS19, was derived from DBA/2J mice and has been shown to accumulate adult β-globin mRNA upon treatment with various chemical inducers such as dimethyl sulfoxide, butyrate, and HMBA (Nudel et al., 1972; Ohta et al., 1976). Recent studies by Brown et al. (1982) have demonstrated that cell lines derived from DBA/2J mice do not express the βh globin genes. In order to determine if the embryonic globin gene was expressed in DS19 cells when treated with 4 mM HMBA and to relate changes in chromatin structure to gene expression, the rates of transcription for the embryonic and adult β-globin genes were analyzed in uninduced and HMBA-induced cells. RNA labeled in isolated nuclei was hybridized to filter-bound DNA fragments containing the embryonic or adult β-globin genes. The rates of transcription are reported in Table III. ϵY3 DNA hybridized the same number of cpm as did pBR322 control DNA and was, therefore, not transcribed in either the induced or uninduced cells. On the other hand, the adult genes were transcribed at a very low rate in uninduced cells and showed significant stimulation upon HMBA induction. Comparing these data with the MNase-digestion data defined three detectable chromatin structures corresponding to an inactive gene, an active gene with a very low transcription rate, and an active gene with a high rate of transcription.

Discussion

The β-globin gene family exists within two distinct chromatin domains in MEL cells as detected by DNase I digestion. The potentially active or actively transcribed β-major and β-minor globin genes reside in a DNase I sensitive domain of at least 25 kb in length. The inactive embryonic and βh globin genes are located within a contiguous but relatively insensitive DNase I domain. The sensitivities of both domains remain at the same level during HMBA-stimulated erythroid differentiation. Our results are consistent with the finding of Miller et al. (1978), who demonstrated that β-globin coding sequences were equally DNase I sensitive in various induced and uninduced MEL cell lines. Our observations bear similarities to the report of Lawson et al. (1982) in which it was shown that the ovalbumin, X, and Y genes that are transcribed at different rates reside within a single, uniform DNase I sensitive domain. Since the β-major and β-minor genes are rarely expressed in the uninduced cell but are coordinately expressed at increased rates during differentiation, the DNase I sensitive chromatin domain appears to define a single unit of transcriptional potential. However, the level of individual gene expression is apparently modulated by factors other than those detected by DNase I sensitivity. The sequestering of genes into a DNase I sensitive domain represents a necessary prerequisite for transcription.

The definition of a DNase I sensitive domain is operational, in that it is dependent upon the digestion conditions used to probe chromatin organization. Under our conditions, approximately 20% of the DNA is rendered acid soluble by 30 min of digestion, and we observe no differences in the level of DNase I sensitivity of the β-major gene and flanking DNA sequences during HMBA induction. One report (Stalder et al., 1980b) has shown that by use of limited DNase I digestion, subdomains of increased sensitivity can be detected within a large DNase I sensitive domain that corresponds to the globin gene coding regions in chicken erythrocyte nuclei. On the other hand, using similar DNase I concentrations, Wood & Felsenfeld (1982) have shown that there is little variation in the DNase I sensitivity between the coding region of the chicken adult β-globin gene and its surrounding DNA sequences except in the region of the 5′ DNase I hypersensitive site. These studies used concentrations of DNase I on an order of magnitude less than the concentrations used in the present study. Recently, we found that the 9.3-kb *Xba*I restriction fragment carrying the β-major gene exhibits a similar increase in DNase I sensitivity in our MEL cell line during differentiation (unpublished results). Therefore, the detection of subtle differences in chromatin structure depends upon the concentration of nuclease used in the digestion study.

Another consideration in a study such as this is the fact that several repetitive sequences exist in the β-globin gene complex (Haigwood et al., 1981), which might interfere with the interpretation of hybridization data. We performed experiments using ϵY3 and β-major cDNA probes and obtained rates of digestion almost identical with those obtained with the GL15.0 and GL7.0 genomic sequences, respectively (Yu et al., 1983). Therefore, the effect of repetitive sequences upon the digestion rates of specific regions of the globin gene complex is insignificant.

While there is little information concerning the actual dimensions of the two DNase I domains defined here, the transition from the sensitive to the insensitive structure occurs rather precisely between the βh3 and β-major gene. The sharpness of this boundary can be compared to the results of Lawson et al. (1982), who demonstrated a gradual transition between the DNase I sensitive domain containing the ovalbumin gene family and the adjacent insensitive flanking chromatin structure. The close proximity of the inactive βh3 gene and the potentially transcribed β-major gene may dictate a more precise boundary. Whereas in the case of the ovalbumin domain, no other known genes reside in close proximity to the active X and Y genes, so a more gradual transition does not interfere with the transcriptional regulation of adjacent genes.

Recently, a DNase I hypersensitive site was found approximately 3560 kbp to the 5′-side of the β-major gene (Yu et al., 1983). This site was barely detectable in uninduced cells but was prominent in induced cells. Although localized very near the boundary between the nuclease-sensitive and insensitive domains, it was actually several hundred base pairs within the DNase I sensitive domain. Whether this hypersensitive site represents a new inducible transcriptional unit remains to be determined.

Micrococcal nuclease defined two large chromatin domains within the β-globin gene family. The sensitive domain contained the β-major and β-minor genes and became more

sensitive to nuclease attack during erythroid differentiation. The major transition of MNase sensitivity was localized between the βh3 and β-major genes. A detailed examination of β-major gene chromatin revealed that changes in MNase sensitivity following induction correlated with the β-major coding sequence and 3′-flanking DNA. Anderson et al. (1983) have shown that during hormone-stimulated transcription of the ovalbumin gene, increased MNase sensitivity was confined to a chromatin region coincident with the 5′- and 3′-ends of the mature ovalbumin mRNA. In MEL cells, Hofer et al. (1982) have shown that the majority of primary β-major gene transcripts extend well beyond the poly(A) site, including the region covered by probe GL0.71. The data presented here, therefore, suggest that selective MNase sensitivity during erythroid maturation corresponds to the primary transcription unit of the β-major gene.

The results presented in this paper are consistent with current models of active-gene chromatin structure. The previous correlations between DNase I sensitivity and active genes, the definition of DNase I sensitive chromatin domains in this report and that of Lawson et al. (1982), and the fact that in nonerythroid cells all genes in the β-globin complex reside in a single inactive chromatin domain suggest that one basic step in gene activation is the sequestering of genes into DNase I sensitive domains. In other words, the lateral movement of a chromatin boundary or boundaries could be an elementary control mechanism of gene expression during development. The structural properties of the DNase I insensitive and sensitive domains and the actual mechanism of how these domains are formed are unknown at the present time.

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Registry No. DNase I, 9003-98-9; micrococcal nuclease, 9013-53-0.

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