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β-Globin Gene Family in Murine Erythroleukemia Cells Resides within Two Chromatin Domains Differing in Higher Order Structure†

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ABSTRACT: The β-globin gene family is organized into two distinct chromatin domains which are digested at significantly different rates by DNase I. We have investigated the possibility that this differential DNase I sensitivity is based upon differences in the higher order structure of chromatin. When nuclei are digested under low ionic strength conditions known to unfold higher order chromatin structures, the differential sensitivity is lost. That is, the relatively DNase I resistant domain, containing the transcriptionally inactive embryonic and β-homologous globin genes, becomes sensitive. When chromatin is recondensed with either $MgCl_2$ or NaCl, thus indicating the higher order coiling of the chromatin fiber, the differential sensitivity is restored. Furthermore, the removal of histone H1, known to be essential for stabilization of higher order chromatin structures, results in the loss of differential DNase I sensitivity. In contrast to the DNase I resistant domain, the transcriptionally active adult β-globin genes show no increase in the rate of digestion when chromatin is unfolded, indicating that this domain may exist as an unfolded nucleosomal chain. The data further suggest that this sensitive domain may be depleted of histone H1.

The primary level of nucleosomal organization is the 10 nm in diameter nucleofilament consisting of nucleosome particles connected by short stretches of linker DNA. Numerous levels of chromatin organization exist between the 10-nm filament and the very compact metaphase chromosome [for a review, see Ris & Korenberg (1979) and McGhee & Felsenfeld (1980)]. Much attention has been focused on the 25-30-nm diameter chromatin fiber which is thought to consist of the 10-nm nucleosomal filament coiled into a close-packed helical array or solenoid (Finch & Klug, 1976; Worcel & Benyajati, 1977; Thoma et al., 1979; Butler & Thomas, 1980; Thoma & Koller, 1981). It has been demonstrated that the conversion of the 30-nm solenoid to the 10-nm filament is a reversible process dependent on the monovalent and/or divalent cation concentration (Thoma et al., 1979; Butler & Thomas, 1980) and the ability of oligonucleosomes to re-form native solenoid structures is strongly correlated with the presence of histone H1 (Butler & Thomas, 1980; Thoma & Koller, 1981).

Nucleases have been widely used to probe chromatin structure, and digestions of nuclei with DNase I have demonstrated that active genes are more accessible to nuclease attack compared to inactive genes (Weintraub & Groudine, 1976). Subsequent studies on the globin genes in chicken erythrocytes (Stalder et al., 1980) and the ovalbumin gene family in hen oviducts (Lawson et al., 1982) have shown that the nuclease-sensitive domains extend well beyond gene coding regions and that these DNase I sensitive domains correlate with transcriptional potential. We have previously demonstrated that the β-globin gene complex in murine erythroleukemia (MEL) cells is organized into two distinct chromatin domains on the basis of their DNase I sensitivity (Smith et al., 1984). The actively expressed β-major and β-minor globin genes are organized into a DNase I sensitive domain while the inactive embryonic and β-homologous (βh) globin genes reside in a relatively insensitive chromatin domain.

In this paper, we have examined the relationship between chromatin domains defined by DNase I and their higher order structure. Specifically, we have determined the effect of (i) altering the ionic strength and (ii) removing histone H1 on the DNase I sensitivity of these two unique globin gene chromatin domains. The findings suggest that the inactive embryonic and βh globin genes reside in a chromatin structure which responds to ionic strength in a manner consistent with reversible transitions in the folding of the 25-30-nm chromatin fiber. In contrast, the active adult globin genes reside in an unfolded structure similar to that of the reported 10-nm chromatin filament. These and other findings are discussed in terms of the primary organization of transcriptionally active and inactive genes in chromatin.

Materials and Methods

Cell Culture. Stock cultures of DS19, a subclone of Friend erythroleukemia cell line 745 derived from DBA/2J mice

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(Ohta et al., 1976), were grown in Eagle's minimal essential media (MEM) supplemented with 100 units of penicillin/mL, 100 μg of streptomycin/mL, and 15% fetal calf serum (Flow Laboratories). Experimental cultures were grown for 72 h to densities between 8×10^5 and 2×10^6 cells/mL. Cells were induced by addition of hexamethylenebis(acetamide) (HMBA) 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.

Isolation and Digestion of Nuclei. Induced DS19 cells were washed 3 times in RSB [10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.6, 10 mM NaCl, and 5 mM $MgCl_2$] and lysed in a Dounce homogenizer. Nuclei were pelleted and washed twice with RSB. Prior to digestion under low ionic strength conditions, nuclei were incubated in 2 mM ethylenediaminetetracetic acid (EDTA) at 4 °C for 15 min, washed twice with 0.25 mM Tris-HCl, pH 7.6, and resuspended at 40 A_{260} units/mL. $MgCl_2$ was added to a final concentration of 0.05 mM, and digestions were performed at 37 °C with 20 units of DNase I (Boehringer-Mannheim) per mL.

Nuclei previously treated with 2 mM EDTA and washed in 0.25 mM Tris-HCl, pH 7.6, were digested in buffers of increased ionic strength in the following manner. Nuclei resuspended at 40 A_{260} units/mL in 0.2 mM Tris, pH 7.6, were supplemented with increasing amounts of $MgCl_2$ at 5-min intervals such that the Mg^{2+} concentration in each step was 0.05, 0.1, 0.15, 0.2, 0.25, 0.5, 1.0, 2.0, and finally 3.0 mM, at which point they were digested with 20 units of DNase I/mL. In a similar fashion, the monovalent cation concentration was raised by adding NaCl such that the concentration was incrementally raised to 1, 5, 10, 20, 30, 40, 50, 60, 80, and 100 mM. Nuclei were digested with 20 units of DNase I/mL in either 40 mM or 100 mM NaCl following the addition of $MgCl_2$ to a final concentration of 0.05 mM.

Extraction of Histone H1. Nuclei were resuspended in citric acid–sodium phosphate buffer, pH 3.0, and made 0.25 M in sucrose, 25 mM in KCl, 1 mM in $CaCl_2$, and 1 mM in $MgCl_2$. Nuclei were mixed gently at 4 °C for 15 min and collected by centrifugation as described by Lawson & Cole (1979). H1 loss was monitored by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis, staining with Coomassie brilliant blue, and by densitometer scanning. The pelleted nuclei were washed 3 times in RSB, resuspended at a final concentration of 40 A_{260} units/mL, and digested at 37 °C as described above.

Filter Hybridizations. DNA from DNase I digested chromatin samples was prepared by treatment with 0.2% SDS and 100 μg of proteinase K/mL at 65 °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, and 1 mM EDTA, and 15 μg of DNA from each sample was bound to nitrocellulose membranes by the methods of Robinson et al. (1982). Restriction fragments were obtained with the appropriate restriction endonucleases and isolated by agarose gel electrophoresis (Roop et al., 1978). 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 × standard saline citrate (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 × SSC, 3 × SSC, and finally 1 × SSC.

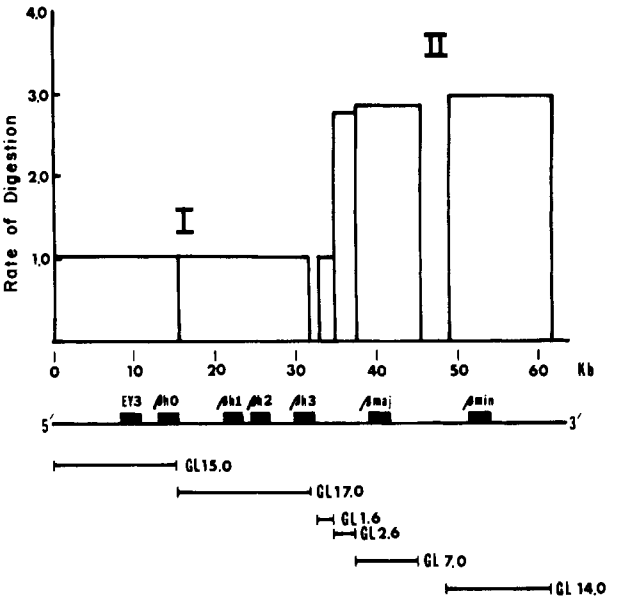

FIGURE 1: Domains of DNase I sensitivity in the globin gene family. The relative rates of digestion for the indicated DNA fragments were obtained from Smith et al. (1984) and are plotted against a map of the globin gene family. In the present study, the fragments GL15.0, GL1.6, GL2.6, and GL7.0 were used as hybridization probes.

Analysis of Nuclease Digestions. Following the hybridization of radioactive probes to nuclease-digested DNA under conditions of probe sequence excess, the amount of filter-bound radioactivity was determined by scintillation counting. The percent hybridization was determined as the fraction of filter-bound cpm for nuclease-digested DNA divided by the cpm bound to undigested total mouse DNA. In order to calculate the rate of digestion, the log of this fraction was calculated and plotted against time. For times of digestion up to 30 min, such plots were linear from which the slope was calculated and the relative rate of digestion determined. Each digestion experiment was performed at lease 2 times. Standard deviations were calculated for each set of relative rates and the average rates reported. The rate of digestion of a particular fragment was independent of probe size (Smith et al., 1984).

Results

Chromatin Domains within the β-Globin Gene Complex Defined by DNase I. In a previous report, the DNase I sensitivity of specific DNA sequences in the mouse β-globin gene complex defined two distinct chromatin domains (Smith et al., 1984). Figure 1 shows that the transcriptionally inactive embryonic and βh globin genes reside in a nuclease-resistant region while the expressed β-major and β-minor globin genes reside in a domain of comparative nuclease snesitivity. The rate of digestion of the sensitive domain was approximately 3 times faster compared to the insensitive domain. The DNA sequences used in the present study as hybridization probes are also shown in Figure 1. The DNA fragments GL15.0 and GL7.0 have been described by Jahn et al. (1980) and Konkel et al. (1978), respectively. The fragments GL1.6 and GL2.6 span the region of transition between the two chromatin domains. Each of these fragments was labeled and used to detect complementary sequences in MEL cell DNA following nuclear digestion with DNase I. In the experiments reported here, MEL cells were treated with HMBA and incubated for 72 h, at which time greater than 95% of the cells were benzidine positive. The two chromatin domains defined by DNase I within the globin gene complex were equally sensitive in both

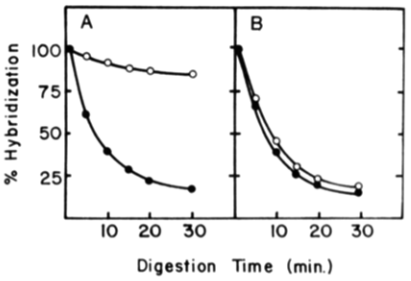

FIGURE 2: DNase I digestion of globin gene chromatin under low ionic strength conditions. Purified nuclei either were digested in RSB (A) or were swollen in EDTA and subsequently digested in 0.25 mM Tris–0.05 mM $MgCl_2$ (B). The purified DNA was hybridized with ^{32}P-labeled GL15.0 (O) or ^{32}P-labeled GL7.0 (●) fragments in probe excess.

fully induced and uninduced cells (Smith et al., 1984), and thus the DNase I sensitive domain represented a primary unit of transcriptional potential, as well as activity.

Effect of Low Ionic Strength on DNase I Digestion of Specific Globin Genes. Purified induced MEL cell nuclei were treated with 2 mM EDTA for 15 min on ice. When examined by light microscopy, these nuclei appeared swollen, and the internal nuclear structures were no longer visible. Such nuclei were gently pelleted and washed at least twice with 0.25 mM Tris-HCl, pH 7.5. After the nuclei were resuspended at 40 A_{260} units/mL, $MgCl_2$ was added to a final concentration of 0.05 mM. This concentration of $MgCl_2$ was sufficiently low to maintain the unfolded chromatin configuration (Zentgraf et al., 1980) while providing sufficient divalent cations for enzyme activity.

Swollen nuclei and control nuclei resuspended in RSB were digested with DNase I, and the amount of DNA complementary to probe GL15.0 or GL7.0 was determined by filter hybridization. Figure 2 shows that under normal digestion conditions (in RSB) the GL15.0 sequence was approximately 3-fold more resistant to DNase I digestion compared to the GL7.0 sequence. However, when nuclei were swollen in EDTA and digested in low ionic strength buffer, the GL15.0 fragment became nearly as sensitive to DNAse I as the GL7.0 fragment. The GL15.0 sequence was digested at least 3 times faster in low ionic strength buffer than in RSB. In contrast, the rate of digestion for the GL7.0 sequence did not change significantly in nuclei treated with 2 mM EDTA (Figure 2B).

DNase I Sensitivity of Globin Gene Chromatin in Recondensed Nuclei. The following experiments utilized the ability of both monovalent and divalent cations to recondense chromatin and examined the nuclease sensitivity of globin gene sequences subsequent to such recondensation. MEL cell nuclei were subjected to treatment in 2 mM EDTA, thoroughly washed in 0.25 mM Tris-HCl, pH 7.5, and resuspended at 40 A_{260} units/mL. $MgCl_2$ was added to a final concentration of 3 mM as outlined under Materials and Methods in order to regenerate higher chromatin structures. During the sequential addition of $MgCl_2$, the nuclei slowly regained their normal morphology as monitored by phase microscopy. Figure 3C shows that there was no detectable loss of nucleosomal proteins during EDTA swelling, washing, and subsequent recondensation in $MgCl_2$.

The recondensed nuclei were digested with DNase I, and hybridization analysis with the GL15.0 and GL7.0 probe sequences was performed. Figure 3 compares the kinetics of digestion of the globin sequences in control nuclei, EDTA-swollen nuclei, and recondensed nuclei. The DNA complementary to GL15.0 was more resistant to DNase I in recondensed nuclei compared to EDTA-swollen nuclei (Figure 3A).

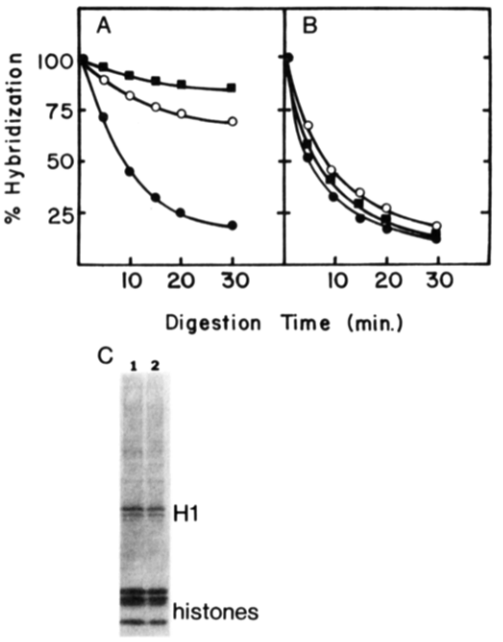

FIGURE 3: Digestion kinetics of globin gene chromatin in $MgCl_2$-recondensed nuclei. DNase I digestions were performed following the recondensation of nuclei in 0.25 mM Tris-HCl, pH 7.5, and 3 mM $MgCl_2$ (O). As controls, nuclei were digested either in RSB (■) or in 0.25 mM Tris-HCl, pH 7.5, and 0.05 mM $MgCl_2$ subsequent to their swelling in EDTA (●). DNA from each digestion series was hybridized with either the GL15.0 (A) or the GL7.0 (B) probe. The proteins from nuclei isolated in RSB (lane 1) or swollen in EDTA and recondensed in $MgCl_2$ (lane 2) were analyzed in SDS–polyacrylamide gels (C).

When compared to control nuclei digested in RSB, the relative rates of digestion indicate that a significant percentage (85%) of DNase I resistance was regained as a result of recondensation with Mg^{2+} ions. Figure 3B shows that the GL7.0 sequence remained sensitive to digestion in both unfolded and recondensed nuclei: a comparison of digestions performed in RSB, in low ionic strength buffers, and in recondensed nuclei yielded nearly identical rates of digestion for this sequence. Thus, the DNase I sensitive domain not only appears to be normally unfolded by this criterion but also apparently lacks the ability to form higher order structures.

Next, we tested the ability of NaCl to recondense chromatin and examined the DNase I sensitivity of the GL15.0 and GL7.0 sequences. Nuclei were swollen as previously described, and NaCl was added to increments to final concentrations of either 40 or 100 mM as outlined under Materials and Methods. When the desired NaCl concentration was reached, $MgCl_2$ was added to a concentration of 0.05 mM, and digestions were performed. There was no apparent loss of specific nucleosomal proteins during recondensation in NaCl (Figure 4C).

Figure 4 compares the kinetics of digestion for the GL15.0 (A) and GL7.0 (B) fragments in NaCl-recondensed nuclei and EDTA-swollen nuclei. In both 40 and 100 mM NaCl-treated nuclei, the GL15.0 sequence became resistant to DNase I as compared to EDTA-swollen nuclei; 100 mM NaCl was more efficient at restoring the normal resistance of the GL15.0 region than was 40 mM NaCl (Figure 4A). The relative rates of digestion indicated a recovery of >90% of normal fragment resistance to DNase I in 100 mM NaCl and 65% in 40 mM NaCl. The resistance of the GL15.0 domain was restored to similar levels when treated with either 100 mM NaCl or 3 mM $MgCl_2$. As with $MgCl_2$, the sensitivity of the GL7.0 region was not significantly altered in 40 mM NaCl and became only

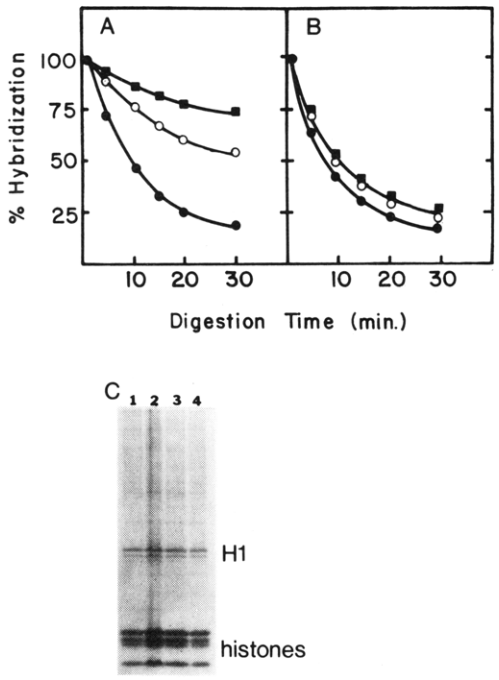

FIGURE 4: Effect of recondensation in NaCl on the digestion of globin gene chromatin. EDTA-swollen nuclei (●) were recondensed in 40 mM (○) or 100 mM (■) NaCl and digested with DNase I. The DNA from each digestion series was hybridized with the GL15.0 (A) or the GL7.0 (B) sequence. The SDS–polyacrylamide gel analysis of chromosomal proteins from control nuclei in RSB (lane 1) or recondensed in 40 mM (lane 3) and 100 mM NaCl (lane 4) is shown in (C). Lane 2 represents proteins from EDTA-swollen nuclei.

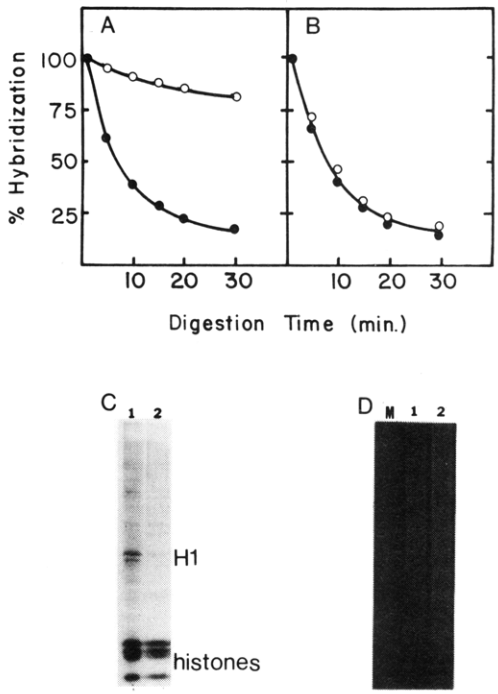

FIGURE 5: DNase I digestion of globin gene chromatin following the removal of histone H1. Histone H1 was removed by the method of Lawson & Cole (1979), and nuclei were digested with DNase I in RSB. The purified DNA in the digestion series was probed with either the GL15.0 (A) or the GL7.0 (B) sequence. Nuclear proteins were analyzed in SDS–polyacrylamide gels following the removal of H1 (C). Nuclei before (○) and after (●) the removal of H1 were digested with micrococcal nuclease, and the purified DNA was separated in 1.5% agarose gels (D). The DNA markers (lane M) were produced by *Hae*III cleavage of ϕX174 DNA and are 1350, 1078, 872, 603, and 310 base pairs in length.

slightly resistant (<10%) to digestion in 100 mM NaCl (Figure 4B).

DNase I Digestion of Globin Genes following the Removal of Histone H1. Histone H1 is intimately involved in the formation and maintenance of chromatin higher order structures (Thoma et al., 1979; Butler & Thomas, 1980; Thoma & Koller, 1981). In the following experiments, the effect of removing histone H1 upon the DNase I sensitivity of globin gene chromatin was examined. Histone H1 was removed by the low-pH method of Lawson & Cole (1979). Figure 5C shows that two successive washings of nuclei in pH 3.0 buffer effectively removed 95% of the histone H1 from the nuclei while no appreciable loss of the core histones H2A, H2B, H3, or H4 was detected. Nuclei treated in this manner appeared normal in the light microscope, and nucleosome structure remained intact as judged by characteristic DNA banding pattern observed in agarose gels following micrococcal nuclease (MNase) digestion (Figure 5D).

The H1-depleted nuclei were washed 3 times in RSB and digested with DNase I. Purified DNA from the digestion series was hybridized to labeled GL15.0 and GL7.0 DNA fragments (Figure 5A,B). The GL15.0 DNA sequence was digested as a significantly greater rate in nuclei where H1 was removed as compared to intact nuclei (Figure 5A). The removal of H1 resulted in a rate of digestion similar to that obtained in EDTA-swollen nuclei. In contrast, the removal of H1 had little effect on the digestion of the GL7.0 DNA sequence as seen in Figure 5B, suggesting the depletion or lack of H1 wihtin the DNase I sensitive domain. Further evidence to support this hypothesis is presented below.

Analysis of Active and Inactive Globin Sequences in Salt-Eluted Nucleosomes. Following digestion by micrococcal nuclease, chromatin can be fractionated according to DNA length and accessory protein content by sequential extraction of nuclei with NaCl (Sanders, 1978). Induced MEL cell nuclei were mildly digested with micrococcal nuclease (<2% acid solubility) and were sequentially extracted with 0.1, 0.2, 0.3, and 0.4 M NaCl. Following extraction, the supernatants were collected, and the DNA was purified by phenol–chloroform extraction. Equal amounts of DNA were bound to nitrocellulose and hybridized with either the labeled GL7.0 or the labeled GL15.0 fragment under conditions of probe excess. The distribution of these sequences is reported in Table I. The active GL7.0 sequence was greatly enriched in the 3.1 M salt eluate such that 57% of the total cpm hybridized was present in this fraction. The remaining cpm were almost equally distributed among the remaining fractions. A similar result was obtained when a cloned cDNA fragment for the β-major gene (Rougeon & Mach, 1977) was used as the hybridization probe (data not shown). In contrast, the inactive GL15.0 sequence was depleted in the 0.1 M salt fraction, and its distribution pattern was very similar to that of bulk DNA.

In order to examine the chromosomal proteins in each salt fraction, MEL cells were grown in the presence of [^{3}H]lysine.

Table I: Distribution of Globin Gene Sequences in Salt-Fractionated Chromatin[a]

	distribution (%)					
	0.1 M	0.2 M	0.3 M	0.4 M	pellet	total cpm
total DNA	10	22	50	10	8	16000
GL7.0	57	9	7	10	17	11200
GL15.0	4	12	60	15	9	12000

[a] Following MNase digestion and Sanders fractionation, purified DNA from each salt fraction and the remaining nuclear pellet was bound to nitrocellulose and hybridized with either the GL7.0 or the GL15.0 probe. The percentages represent the fraction of total cpm hybridized in each series.

Table II: Summary of DNase I Digestion Data[a]

treatment of nuclei	digestion buffer	relative rate of digestion	
		GL15.0	GL7.0
(a) RSB	RSB	0.010±.001[b]	0.030±.003[b]
(b) 2 mM EDTA, 15 min, 4 °C	0.25 mM Tris-HCl, pH 7.5, 0.05 mM $MgCl_2$	0.029	0.031
(c) 2 mM EDTA, 15 min, 4 °C	0.25 mM Tris-HCl, pH 7.5, 3.0 mM $MgCl_2$	0.011	0.029
(d) 2 mM EDTA, 15 min, 4 °C	0.25 mM Tris HCl, pH 7.5, 40 mM NaCl, 0.05 mM $MgCl_2$	0.015	0.029
(e) 2 mM EDTA, 15 min, 4 °C	0.025 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.05 mM $MgCl_2$	0.010	0.028
(f) pH 3.0 buffer	RSB	0.029	0.030

[a] All nuclei were isolated in RSB and subjected to the conditions indicated. The rates of digestion represent the slopes obtained as outlined under Materials and Methods. [b] Standard deviation.

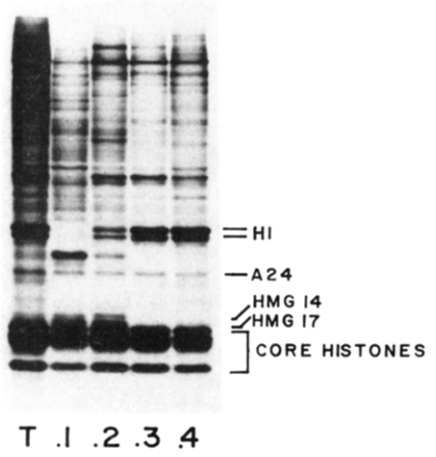

FIGURE 6: Salt fractionation of nuclear proteins. SDS–polyacrylamide gel analysis of chromosomal proteins sequentially eluted in 0.1, 0.2, 0.3, and 0.4 M NaCl. The first lane shows total (T), unfractionated MEL cell nuclear proteins.

Nuclei were digested with MNase, and the labeled proteins from each salt eluate were analyzed in SDS–polyacrylamide gels (Figure 6). The 0.1 M salt fraction contained the core histones and was enriched in high mobility group (HMG) proteins 14 and 17 which migrate more slowly than histone H3. However, in the 0.2 M NaCl eluate, the HMG proteins were similarly enriched while the GL7.0 sequence was not. There was also an enrichment of an unidentified protein in the 0.1 M fraction which migrated between the H1 doublet and the A24 band. Notably, the 0.1 M eluate contains no H1. Previous reports have shown that the 0.1 M NaCl fraction contains only mononucleosomes lacking H1 (Annunziato et al., 1981). The 0.2, 0.3, and 0.4 M salt fractions contained histone H1 as well as the four core histones. The lack of any detectable histone H1 and the insignificant enrichment of the GL7.0 sequence in the 0.1 M salt fraction support the hypothesis that histone H1 is greatly depleted in the DNase I sensitive chromatin domain.

Accurate Refolding of the Chromatin Boundary with $MgCl_2$ or NaCl. As shown in Figure 1, the DNA fragments GL1.6 and GL2.6 reside adjacent to but on opposite sides of the boundary region between the DNase I sensitive and insensitive chromatin domains. In order to determine if the two domains refold accurately in relation to the position of the globin genes, the GL1.6 and GL2.6 sequences were used to probe chromatin refolded in the presence of Mg^{2+} ions or Na^+ ions.

MEL cell nuclei were swollen in the presence of 2 mM EDTA. The sample was split, and half of the nuclei were refolded with increasing concentrations of $MgCl_2$ to a final concentration of 3 mM. The remaining nuclei were treated with increasing amounts of NaCl to a final concentration of 100 mM as previously described. Figure 7A shows that following unfolding in EDTA the GL1.6 sequence became more sensitive to digestion. However, approximately 85% of normal

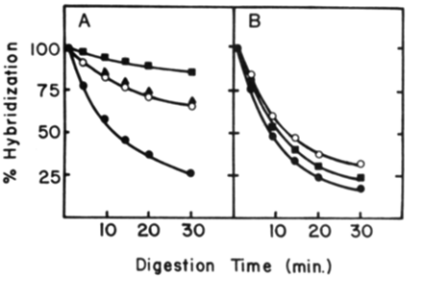

FIGURE 7: Accurate refolding of chromatin domains. EDTA-swollen nuclei (●) were recondensed in the presence of $MgCl_2$ (○) or 100 mM NaCl (▲) and digested with DNase I. Control nuclei were digested in RSB (■). The DNA from each digestion series was probed with either the GL1.6 (A) or the GL2.6 (B) DNA sequence.

resistance was restored by refolding with $MgCl_2$. Between 85% and 90% normal resistance was restored with 100 mM NaCl. On the other hand, as shown in Figure 7B, the GL2.6 sequence was not significantly affected either by EDTA treatment or by refolding with $MgCl_2$ or NaCl, remaining relatively sensitive to DNase I attack in all cases. Therefore, during swelling and subsequent refolding, the position of the original chromatin domains was restored with accuracy.

Discussion

It is clear that active genes reside in chromatin structures which are relatively sensitive to DNase I compared to inactive genes [for reviews, see Mathis et al. (1980) and Cartwright et al., (1983)]. However, the actual basis for the preferential sensitivity is poorly understood. The results of this paper suggest that one elementary difference between DNase I sensitive and insensitive chromatin is a difference in the higher order coiling of the nucleosomal filament. In order to facilitate discussion of the results, the rates of digestion have been calculated for each set of experiments and are shown in Table II. Also, in Figure 1, chromatin domains I and II are defined with respect to transcriptional activity and the position of the β-globin genes in MEL cells.

When chromatin is subjected to low ionic strength conditions, the 25–30-nm fiber unfolds to yield the 10-nm unit filament of nucleosomes (Thoma et al., 1979; Butler & Thomas, 1980). Under low ionic strength conditions, the DNase I resistant domain I (Figure 1) was digested at a rate nearly 3 times faster relative to normal digestions performed in RSB [compare (a) and (b) of Table II]. This rate of digestion was nearly the same as that found for domain II containing the active adult globin genes (Figure 1). When swollen nuclei were recondensed in 3 mM $MgCl_2$, the chromatin in domain I regained most of its original resistance to DNase I (Table IIc). The resistance of domain I was, therefore, restored under conditions which are known to refold 10-nm chromatin fibers into the 30-nm form (Thoma et al., 1979; Butler & Thomas, 1980; Zentgraf et al., 1980; Thoma & Koller, 1981).

The 10-nm nucleosomal filament has also been shown to refold with increasing concentrations of sodium ions (Thoma et al., 1979; Butler & Thomas, 1980). When EDTA-swollen nuclei were recondensed in 100 mM NaCl, domain I chromatin regained its resistance to DNase I (Table IIe) while domain II remained at the same level of sensitivity. At an intermediate concentration of 40 mM NaCl, the resistance of domain I was only partially restored. This observation is in agreement with two previous reports. Thoma et al. (1979) and Zentgraf et al. (1980) have shown that at 40 mM NaCl the solenoid is at an intermediate level of condensation having three to four nucleosomes per turn compared to the fully condensed fiber with six to eight nucleosomes per turn in 100 mM NaCl. Thus, DNase I sensitivity is proportional to the extent of chromatin folding.

It has been suggested that histone H1 is essential for the stability of higher order chromatin structures [for a review, see Mathis et al. (1980)]. H1 binds at the point of DNA entry and exit on the nucleosome (Thoma et al., 1979; Boulikas et al., 1980; Allan et al., 1980), and it protects a small amount of linker DNA from nuclease digestion (Noll & Kornberg, 1978). When greater than 90% of histone H1 was selectively removed from MEL cell nuclei, chromatin domain I became sensitivie to DNase I digestion (Table IIf). In this case as in the experiments where the chromatin was unfolded in EDTA, domain I became as sensitive as domain II [compare (b) and (f) of Table II]. These results and the finding that the GL15.0 sequence was present in H1-containing chromatin are consistent with the interpretation that under conditions of physiological ionic strength (about 150 mM) the transcriptionally inactive domain contains histone H1 and is in the form of a higher order compact nucleosomal fiber.

Under all of the conditions examined, the chromatin domain containing the active adult β-globin genes was digested by DNase I at nearly the same rate (Table II). The heightened digestibility of this region suggests that it is more accessible to DNase I, perhaps due to a localized unfolding of the 30-nm chromatin fiber. This interpretation is consistent with our finding that treatments which unfold the 30-nm fiber do not increase the rate of digestion of domain II. Even during the recondensation of nuclei with Mg^{2+} or Na^{+} domain II did not lose its DNase I sensitivity, suggesting that some inherent property of this region may prevent its higher order coiling.

When histone H1 was removed from nuclei, the digestibility of the active domain (II) was not altered. In addition, there was a significant enrichment (>5-fold) of the GL7.0 sequence in the 0.1 M NaCl fraction which did not contain histone H1. A similar enrichment of active sequences has been reported by Bloom & Anderson (1978), and similar active chromatin fractions have been shown to be free of H1 (Tata & Baker, 1978; Goldknopf et al., 1978), although others have reported active chromatin fractions to contain histone H1 (Gottesfeld & Butler, 1977; Davie & Candido, 1978). The depletion of H1 may explain certain characteristics of active chromatin: (i) the low compaction ratio of active chromatin observed in the electron microscope [for a review, see Mathis et al. (1978))]; (ii) the preferential senstivity to micrococcal nuclease; and (iii) the increased solubility of active chromatin in NaCl (Gottesfeld et al., 1974; Levy-Wilson et al., 1979).

H1-depleted chromatin has been shown to undergo slight but unorganized refolding under conditions of increased ionic strength although not into well-defined structures (Thoma et al., 1979). The data in Figure 5 show, however, that H1-depleted inactive chromatin was digested in RSB (3 mM Mg^{2+}) at a rate similar to that of the active domain in normal or H1-depleted chromatin [Table II, compare (a) and (f)]. Thus, minor refolding of H1-depleted domain I was not detectable with DNase I, suggesting that it is the organized compaction of nucleosomes into the 30-nm fiber that protects the DNA against nuclease attack.

The higher order compaction and presence of H1 in inactive chromatin vs. the H1-depleted, extended form of active chromatin may explain much of the differential nuclease sensitivity between the two regions. However, other factors are probably involved. For example, DNase I sensitivity of active regions is abolished by extraction of non-histone proteins (NHP) at ionic strengths that do not remove H1 and is restored by reconstitution of NHP (Weisbrod & Weintraub, 1979; Gazit et al., 1980; Reeves & Chang, 1983). On the other hand, the presence or absence of NHP does not affect the H1-dependent ability of chromatin to undergo reversible unfolding–refolding transitions (Thoma & Koller, 1981).

At the ionic strength used (100 mM) to fully recondense chromatin, H1 has been shown to exchange (Caron & Thomas, 1981); yet, we have consistently observed specificity not only in the refolding of region I chromatin but also in the lack of refolding of region II chromatin. It may prove significant that intact nuclei, rather than soluble chromatin fragments, were used in the study of these large domains, since others have shown higher order structure formation to be size dependent (Butler & Thomas, 1980). Since exchange of H1 would be expected to cause region II chromatin to condense, it is apparent either that exchange under our conditions is low or that in intact nuclei other factors contribute to the capacity of nucleosomes to bind H1.

In summary, the transcriptionally inactive embryonic and βh globin genes reside in a chromatin domain containing histone H1, which is resistant to DNase I and may represent the 30-nm chromatin fiber. In contrast, the adult β-globin genes reside in a chromatin domain which is depleted in histone H1, is sensitive to DNase I digestion, and may represent the unfolded 10-nm nucleosomal filament. Since the entire globin gene family is resistant to DNase I in nonerythroid cells (Smith et al., 1984), the localized unfolding of the 30-nm chromatin fiber possibly represents one step in gene activation and may occur early in the development of erythroid cells. Thus, the concept of chromatin as a mosaic of condensed inactive regions alternating with unfolded active regions appears to hold for pol II genes as well as for rRNA genes (Pruitt & Grainger, 1981). A primary difference in organization may exist, however, since the nontranscribed spacer DNA in rRNA genes is condensed, while the nontranscribed flanking regions of pol II genes are not (Stalder, 1980; Lawson et al., 1982; Wood & Felsenfeld, 1982; Smith et al., 1984).

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