Reversible Changes in the Nucleosomal Organization of a Human H4 Histone Gene during the Cell Cycle†

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ABSTRACT: The organization of nucleosomes associated with a cell cycle regulated human H4 histone gene was examined in synchronized HeLa S3 cells. At various times during the cell cycle, nuclei were digested with micrococcal nuclease, and the nucleosomal pattern of the gene was obtained by Southern blot analysis using radiolabeled human histone H4 gene probes. We have detected reversible changes during the cell cycle in the chromatin structure of this gene, as reflected by the shortening of the nucleosomal spacing after replication and the peak of transcription. This variation is also observed when DNA and protein syntheses are inhibited. By using a probe that comprises 250 base pairs (bp) of the coding region and 240 bp of the 5′ end of the gene, containing the promoter and DNase I sensitive sequences, we also have observed a general disruption of the nucleosomal organization, which is reflected by a degeneration of the characteristic nucleosomal ladder produced by micrococcal nuclease digestion. This modification coincides with the replication and active transcription of the gene (early S phase), which recovers its regular nucleosomal appearance when both processes have been completed, although the nucleosome linker length is shortened. When the probe utilized comprises the distal 3′ end of the gene, there is no disruption of the nucleosomal pattern, but the linker region also exhibits a shortened length. A non-cell cycle regulated gene (β-globin) does not exhibit such modifications in any of the situations analyzed. The observed variations in nucleosomal spacing and in the regular nucleosomal pattern may be functionally related to modifications in DNase I and S1 sensitivity in the 5′ end of the H4 gene during S phase [Chrysogelos, S., Riley, D. E., Stein, G., & Stein, J. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 7535]. Together, these results suggest that there is a sequential remodeling of chromatin structure of a cell cycle dependent human H4 histone gene that correlates with expression.

Alterations in nucleosomal organization that are associated with modifications in gene expression have been extensively examined during the past several years. Changes both in total chromatin [for references, see Eissenberg, et al. (1985), Reeves (1984), Igo-Kemenes et al (1982), Tsanev (1983), Cartwright et al. (1982), and Weisbrod (1982)] and in specific genes (Smith et al., 1983; Murray & Kennard, 1984; Lohr, 1983, 1984; Gottschling, 1983) have been observed. From these data, there emerges a general consensus about the higher accessibility of transcriptionally active chromatin to nuclease digestion and some features in the overall organization of active chromatin that make it distinguishable from inactive chromatin (Weintraub & Groudine, 1976; Garel & Axel, 1976; Reeves, 1984). Modifications in the nucleosomal repeat length have also been described, although the exact nature of these changes has not been established. In some cases, it becomes shortened (Savic et al., 1981; Berkowitz & Riggs, 1981; Chambers et al., 1983), but longer repeats have also been reported in the chromatin structure of active compared with inactive genes (Smith et al., 1983). Even the absence of detectable changes has been described (Gottesfeld & Melton, 1978).

Alterations in nucleosomal organization have also been observed in conjunction with DNA replication. There is a general agreement that the internucleosomal linker shows a shortened length in newly replicated chromatin (D'Anna & Prentice, 1983; Annunziato & Seale, 1982).

Cell cycle dependent human histone genes are replicated during the first third of the S phase of the HeLa cell cycle (Iqbal et al., 1984), and it is also in this period that these genes reach their maximal level of transcription (Plumb et al., 1983; L. L. Baumbach et al., submitted for publication). Experiments measuring the in vivo incorporation of [^{3}H]uridine into histone mRNAs from synchronized HeLa cells showed maximal incorporation occurring between the first and second hours of S phase (Plumb et al., 1983). Similar results were obtained from in vitro nuclear transcription experiments (L. L. Baumbach et al., submitted for publication). Recent results demonstrate the presence of DNase I and S1 nuclease-sensitive sites in a cell cycle dependent human H4 histone gene designated pF0108A (Chrysogelos et al., 1985). The nuclease-sensitive sites are located in the 5′-flanking segment of this H4 histone gene in the region where sequences that influence the extent of transcription and site-specific initiation reside (Sierra et al., 1983). Both the DNase I sensitive and S1-sensitive sites appear to be modified during the S phase of the cell cycle.

To examine further modifications in the chromatin structure of the pF0108A H4 histone gene that may be related to its expression, we have analyzed the nucleosomal pattern of this gene during the cell cycle. For comparison, a parallel study was carried out on the β-globin gene, which replicates later during S phase (Lo et al., 1980) and is not expressed in HeLa cells (Marashi et al., 1986). Here, our goal was to distinguish between changes in chromatin structure that may be related to replication and transcription of the pF0108A histone gene.

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We have additionally analyzed the nucleosome pattern of this H4 histone gene following inhibition of DNA and protein synthesis since such treatments alter histone mRNA stability but have no effect on histone gene transcription (Stein et al., 1984; Baumbach et al., 1984) and have been shown to affect the nucleosomal spacing of newly replicated chromatin (D'Anna & Prentice, 1983; D'Anna & Tobey, 1984; Leffak, 1983).

From these analyses, we have detected significant variations during the cell cycle in the length of the linker region of the human histone H4 gene chromatin and a general disruption of the nucleosomal organization of the gene in early S phase. These modifications are not shared by the chromatin of the β-globin gene, the properties of which are indistinguishable from HeLa cell total chromatin.

Materials and Methods

Cell Culture, Synchronization, and Treatments. HeLa S3 cells were grown in suspension culture in Eagle's minimal essential medium supplemented with 7% calf serum. Exponentially growing cells were synchronized by two successive treatments with 2 mM thymidine (Stein & Borun, 1972).

DNA synthesis rates were monitored by measuring the incorporation of radioactivity into 5% trichloroacetic acid precipitable material after pulse labeling of duplicate samples for 30 min with [^{3}H]thymidine (Shephard et al., 1982).

Samples of synchronized cells were harvested at different points during the cell cycle and processed as detailed below. For each selected time, identical volumes of the cell suspension were treated for 1 h with either 1 mM hydroxyurea or 10 μg/mL cycloheximide.

Isolation of Nuclei and Micrococcal Nuclease Digestion. Cell samples from different cell cycle times were lysed in reticulocyte standard buffer [RSB: 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, 15 mM NaCl, and 1.5 mM $MgCl_2$] containing 0.1% Triton X-100. Nuclear pellets were washed several times in RSB and resuspended in RSB at a concentration of 20 A_{260} units/mL. $CaCl_2$ was added to the nuclear suspension to a final concentration of 1 mM, and the nuclei were incubated for 1, 2, 5, or 10 min at 37 °C with 100 units/mL micrococcal nuclease (Boehringer). Digestion was stopped by the addition of ethylenediaminetetraacetic acid (EDTA) to a final concentration of 5 mM. DNA was isolated from the digested nuclei by treatment with 0.5% sodium dodecyl sulfate (SDS) and 50 μg/mL proteinase K for 10 h at 37 °C followed by two extractions with phenol/chloroform/isoamyl alcohol (25:24:1), two extractions with chloroform/isoamyl alcohol (24:1), and precipitation with 3 volumes of ethanol at −70 °C.

Electrophoresis, Blotting, and Hybridization. Samples containing 15 μg of DNA were fractionated electrophoretically in 2% agarose gels (25 × 21 cm) at 65 V for 9 h. The DNA was transferred to nitrocellulose filters (0.2-μm pore diameter) according to the method of Southern (1975) and hybridized to the following probes: *Eco*RI/*Hin*dIII insert from pF0108A subclone [H4 insert, 1.85 kilobases (kb)] (Figure 2); *Nco*I/*Eco*RI fragment from pF0108A subclone (H4 coding region and 5′ end, 0.49 kb) (Figure 5A); *Xba*I/*Pst*I fragment from pF0108A subclone (3′ end, 0.41 kb) (Figure 6); *Pst*I insert from *β-Pst* subclone (β-globin insert, 4.2 kb) (Figure 3); *Bam*HI/*Nco*I fragment from BSB subclone (β-globin coding region, 0.428 kb) (Figure 5B). The fragments were eluted from agarose gels and nick-translated (Maniatis et al., 1975) to a specific activity of 10^8 cpm/μg of DNA (5 × 10^6 cpm mL^{-1}). Hybridization was performed at 67 °C in 4 × SSC, 5 × Denhardt's solution [1 × Denhardt's is 0.02% ficoll/0.02% poly(vinylpyrrolidone)], 0.002% bovine serum albumin (BSA), and 0.1 mg/mL *Escherichia coli* DNA. Blots were washed in 0.1 M potassium phosphate (pH 7.4) for 30 min and 1 × SSC/0.6% SDS for 60 min at 60 °C.

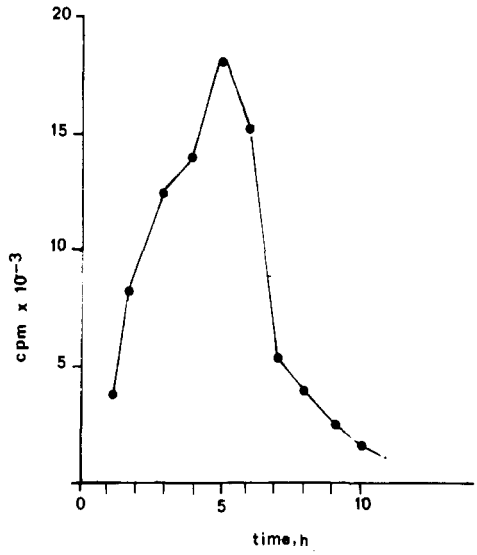

FIGURE 1: Incorporation of [^{3}H]thymidine in the HeLa cell population after release from a double thymidine block. At each time point, duplicate 2-mL aliquots of cells were incubated for 30 min with 4 μCi of [^{3}H]thymidine at 37 °C. Radioactive DNA was precipitated with 10% trichloroacetic acid and collected on type HA Millipore filters. Filters were solubilized in cellosolve, and radioactivity was measured by scintillation spectrometry (Shephard et al., 1982).

Repeat Length Measurements. Migration distances of bands observed in the negatives of ethidium bromide stained gels or in autoradiographs were obtained from densitometric scans. The lengths of DNA fragments were calculated from the calibration curve determined by the markers (λ DNA digested with *Eco*RI/*Hin*dIII and pBR322 digested with *Hin*fI). At least six groups of data from each cell cycle time in untreated, hydroxyurea-treated, or cycloheximide-treated cells were plotted individually by using a least-squares regression analysis, and their repeat lengths were calculated from the slopes, as described by Smith et al. (1983). Means from the different cell cycle points examined were compared as follows: (1) Repeat lengths from different cell cycle times with the same treatment were compared among themselves by analysis of variance, obtaining the Fisher F value. (2) Repeat lengths from a given cell cycle time were compared with those from other DNA segments or total chromatin by using a Student's t test.

All the statistical calculations were performed by using EPISTAT statistical computer programs written by Tracy L. Gustavson (Round Rock, TX).

Results

Changes in the Nucleosomal Spacing of a Human H4 Histone Gene during the Cell Cycle. Initially, the organization of nucleosomes associated with a cell cycle regulated human H4 histone gene was examined during the cell cycle in HeLa S3 cells. Cells were synchronized by two cycles of 2 mM thymidine block, and as shown in Figure 1, DNA synthesis was monitored by [^{3}H]TdR incorporation into trichloroacetic acid (Cl_3CCOOH)-precipitable material. Nuclei were isolated from synchronized HeLa cells at 1 h (early S phase and the time of maximal histone gene transcription), 5 h (the peak of S phase and maximal cellular levels of histone mRNAs), and 10 h (mitosis–G1 and the period of minimal histone mRNA levels) after release from thymidine block. These nuclei were

digested with micrococcal nuclease, and the resulting fragments were separated by electrophoresis in 2% agarose gels, blotted to nitrocellulose filters, and hybridized to a number of nick-translated DNA probes corresponding to various regions of this H4 histone gene and its flanking sequences. The lengths of the nucleosomal repeats were calculated from densitometric tracings of the resulting autoradiograms by linear regression analysis of oligomer size vs. nucleosome number (Smith et al., 1983).

An autoradiograph of a Southern blot of micrococcal nuclease digested DNA samples at 1, 5, and 10 h is shown in Figure 2. The probe was ^{32}P labeled and represented the complete pF0108A extended genomic region, containing the H4 histone gene and 240 nucleotides of upstream- and 1300 nucleotides of downstream-flanking sequences. There are clear differences in the nuclease accessibility of this gene during the cell cycle, with high accessibility to digestion after 2 min at 1 and 5 h, while at 10 h the DNA remains undigested after 2-min incubation with the enzyme. For calculating nucleosomal repeat lengths, we used samples with a similar level of digestion, which was estimated according to the relative amount of the different oligomer fractions in the densitometric scans. For the 1- and 5-h calculations, we used those samples which were incubated for 5 min with the nuclease, and for 10 h, the corresponding samples after 10 min of incubation with micrococcal nuclease were used. In this way, we tried to avoid possible variations in the nucleosomal repeat that may reflect differences in the extent of nuclease digestion (Annunziato & Seale, 1982).

The nucleosomal repeat length during the cell cycle apparently begins to shorten by 1 h (early S phase) (175 ± 7 bp), reaches its shortest value at 5 h (mid S phase) (168 ± 3 bp), and changes to a longer length by 10 h (mitosis–G1 phase) (187 ± 8 bp) (Table I). We accepted as a significant difference in the means those values which showed a level of certainty between 95% and 99%. At 5 h, the H4 gene exhibited obvious differences, when it was compared either with the total chromatin population at the same cell cycle time or with itself in other cell cycle situations (Table I), while total chromatin showed a progressively longer repeat length with a maximal value of 200 ± 4 bp at 10 h. The size of the monomer remained similar in all cases (155–165 bp) under the moderate digestion conditions we used.

To further assess the functional significance of modifications in nucleosome repeat length, we examined this parameter of pF0108A histone gene chromatin structure following inhibition of DNA or protein synthesis. The rationale was that such inhibition modifies histone mRNA stability but has little effect on histone gene transcription and hence would not be expected to influence nucleosome repeat length if it is associated with transcriptional activity. When HeLa cells were treated with 1 mM hydroxyurea or 10 μg/mL cycloheximide for 1 h on each selected time (1, 5, or 10 h), no changes were observed in the nucleosome repeat length of the bulk chromatin (Table I). Similarly, HU treatment and cycloheximide treatment did not significantly influence the cell cycle dependent changes observed in the nucleosomal organization of the pF0108A extended H4 genomic region. As indicated in Figure 4 and Table I, variations in the nucleosome repeat of this gene that occur at 1 h (early S), 5 h (mid S), and 10 h (mitosis–G1) are evident both in control cells and in cells where DNA replication or protein synthesis was inhibited for 1 h.

It should be noted that chromatin from cycloheximide-treated mitotic–G1 cells (10 h) was not digested by micrococcal nuclease even after 10 min of incubation (not shown),

Table I: Nucleosome Repeat Lengths (Base Pairs) during the HeLa Cell Cycle for Total Chromatin, H4 Histone, and β-Globin Genes[a]

	untreated			hydroxyurea			cycloheximide		
	1 h	5 h	10 h	1 h	5 h	10 h	1 h	5 h	10 h
total chromatin	184 ± 4 (n = 8)	190 ± 7 (n = 8)	200 ± 4 (n = 8) $p < 0.01$[d]	183 ± 6 (n = 8)	188 ± 5 (n = 8)	198 ± 6 (n = 4) $p < 0.05$[d]	181 ± 5 (n = 8)	186 ± 7 (n = 8)	199 ± 7 (n = 4) $p < 0.01$[d]
H4 gene	175 ± 7 (n = 9)	168 ± 3 (n = 9) $p < 0.001$[b] $p < 0.001$[c] $p < 0.01$[d]	187 ± 8 (n = 8)	176 ± 7 (n = 6)	164 ± 8 (n = 6) $p < 0.001$[b] $p < 0.005$[c] $p < 0.001$[d]	200 ± 3 (n = 5)	181 ± 9 (n = 7)	163 ± 6 (n = 9) $p < 0.001$[b] $p < 0.001$[c] $p < 0.001$[c] $p < 0.001$[d]	
β-globin gene	187 ± 5 (n = 6)	192 ± 10 (n = 6)	190 ± 9 (n = 5)	179 ± 4 (n = 6)	181 ± 5 (n = 6)	188 ± 5 (n = 4)	178 ± 3 (n = 7)	181 ± 6 (n = 5)	

[a] Nucleosomal repeat lengths measured for the total chromatin population, histone H4, and β-globin genes. Measurements were taken at three different times of the cell cycle in untreated, hydroxyurea-treated or cycloheximide-treated HeLa cells. 1, 5, and 10 h indicate 1, 5, and 10 h, respectively, after release from double thymidine block. Values are given ± the mean standard deviation. n indicates sample size. Cycloheximide-treated chromatin from H4 and β-globin genes remained undigested at 10 h (mitosis–G1) after 10-min incubation with micrococcal nuclease. Probabilities (p) are given only in the cases where significant differences were detected. [b] Significant difference compared to the total chromatin population. [c] Significant difference compared to the β-globin gene. [d] Significant difference when samples under the same treatment were compared among themselves.

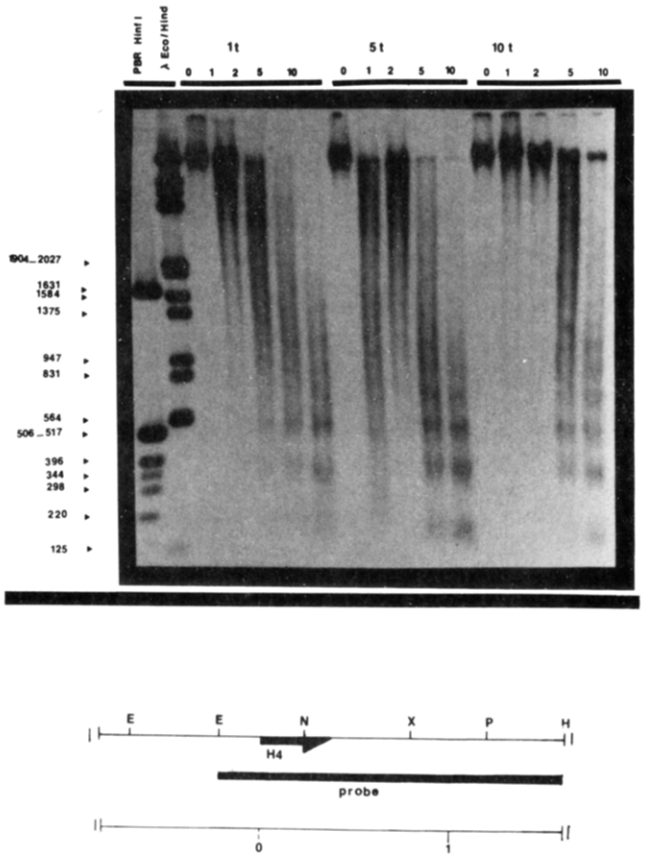

FIGURE 2: Nucleosomal pattern of the pF0108A human H4 histone gene and flanking sequences. Synchronized HeLa cells were selected at 1 h (early S phase), 5 h (mid-S phase), or 10 h (mitosis–G1 phase) after release from a double thymidine block, and nuclei were isolated and digested with micrococcal nuclease (100 units/mL) for 1, 2, 5, and 10 min at 37 °C. Purified DNA was electrophoresed, blotted, and hybridized to the pF0108A insert containing the H4 histone gene. Molecular weight markers were electrophoresed in the two left-most lanes, and their sizes are indicated on the left side of the figure. The lower part of the figure shows a schematic diagram of the gene and the nick-translated probe used for the hybridization. The bar at the bottom indicates the length of the fragment in kilobases. E = *Eco*RI, N = *Nco*I, X = *Xba*I, P = *Pst*I, and H = *Hin*dIII.

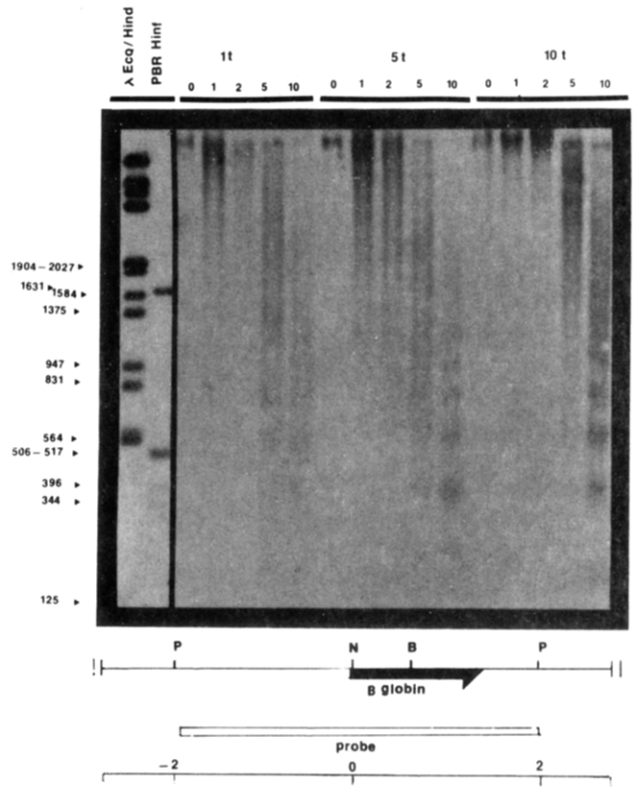

FIGURE 3: Nucleosomal pattern of the human β-globin gene in HeLa cells. HeLa cell samples were processed as indicated in Figure 2, and blots were hybridized to the *β-Pst* insert, containing the β-globin gene and flanking regions (bottom). Molecular weight markers are indicated to the left of the upper part of the figure. P = *Pst*I, N = *Nco*I, and B = *Bam*HI.

suggesting that this chromatin is highly condensed following inhibition of protein synthesis. Additionally, during early S phase (1 h), the nucleosomal organization is somewhat less pronounced when protein synthesis is blocked. The last observation is consistent with previously published results (Annunziato & Seale, 1982; Pospelov et al., 1983).

β-Globin Gene Does Not Exhibit Cell Cycle Dependent Changes in Chromatin Structure. The modifications detected in the nucleosomal repeat of the H4 gene during the cell cycle prompted us to analyze the nucleosomal organization of the β-globin gene for comparison. The β-globin gene is not transcribed in HeLa cells (Marashi et al., 1986), and it replicates late during the S phase of the cell cycle (Lo et al., 1980). The same Southern blots of micrococcal nuclease digested cell cycle samples shown in Figure 2 were rehybridized with a ^{32}P-labeled β-globin gene probe (Figure 3). In the autoradiograph shown in Figure 3, the nucleosomal ladder exhibited by this gene at 1 h, 5 h (where presumably its replication occurs), and 10 h can be observed. Our results indicate that the nucleosomal repeat length of the human β-globin gene does not vary significantly during the cell cycle (Table I) and that this parameter corresponds with measurements for total chromatin. The nucleosome repeat length of the globin gene was not modified by inhibition of DNA or protein synthesis (Table I, Figure 4). As with the H4 gene, micrococcal nuclease was unable to digest the β-globin chromatin after 10 min of incubation when protein synthesis was inhibited during the mitosis–G1 period. It is also interesting to note the regularity of the densitometric scans for the β-globin gene compared with the H4 gene (Figure 4), which exhibited an unequal distribution of nucleosome fragments in all the scans. The irregularities of the H4 nucleosome profiles may be closely related to the heterogeneity present in the extended H4 genomic region, as evidenced by the hybridization with smaller regions contained within (see below).

Nucleosomal Organization of the H4 Gene Is Disrupted during Early S Phase. We also analyzed the nucleosomal pattern in the area of the histone H4 gene which comprises the initial 250 bp of the mRNA coding region and 240 nucleotides of 5′-flanking sequences containing the promoter and cell cycle modified DNase I and S1 nuclease-sensitive sites (Figure 5A). When Southern blots of micrococcal nuclease digested chromatin from various points during the cell cycle were hybridized with this probe, we did not detect a regular nucleosome ladder in the 1-h sample, indicating that the nucleosomal organization of this region is disrupted during early S phase. This point corresponds to the time of replication and maximal transcription for this gene. At 5 and 10 h, when replication of the pF0108 H4 histone gene is completed and transcription is reduced, the regular nucleosome pattern is restored, although the repeat length measured in three samples was significantly shorter at 5 h (170 ± 5 bp) than at 10 h (184 ± 2 bp). We compared these results with the analogous region in the β-globin gene and observed the characteristic nucleosomal ladder at all the cell cycle times analyzed (Figure 5B).

To determine if the disruption of nucleosomal organization during S phase extends beyond the 3′ end of the H4 histone

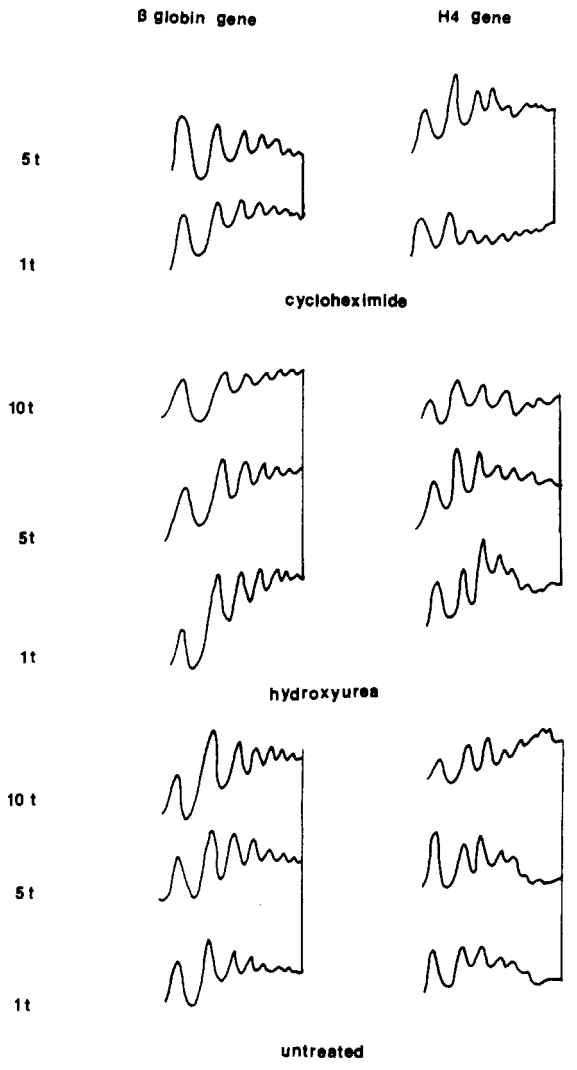

FIGURE 4: Densitometric scans of the electrophoretic patterns for the human H4 histone and β-globin genes after digestion of nuclei with micrococcal nuclease. The scans were generated from autoradiograms of Southern blots hybridized with appropriate nick-translated probes. The numbers on the left indicate the hours after release from thymidine block. Hydroxyurea and cycloheximide treatments were for 1 h as described under Materials and Methods.

gene, we hybridized the cell cycle samples to a probe derived from the distal 3′ end of the H4 coding region. Our results, shown in Figure 6, indicate the presence of a regular nucleosome ladder at all the cell cycle points examined. There were, however, visible variations in the accessibility of this sequence to micrococcal nuclease with the highest accessibility at 5 h as reflected by increased digestion after 2 min of incubation with the enzyme (Figure 6). As observed with the other H4 histone probes, the nucleosomal repeat length of the 3′-flanking region at 5 h (165 ± 6 bp) was significantly shorter than at 10 h (183 ± 2 bp) and showed a value of 176 ± 6 bp at 1 h.

Taken together, our results for the histone H4 and β-globin genes suggest that the general disruption of nucleosome structure in the 5′-flanking region and mRNA coding region of the pF0108A H4 histone gene is more closely correlated with cell cycle dependent modifications in transcription than with replication.

Discussion

In this paper, we present evidence for modifications during the HeLa cell cycle in the nucleosomal organization of a human H4 histone gene that is expressed in a cell cycle dependent manner. Such modifications include the shortening of the nucleosomal spacing (Table I) (168 ± 3 bp) in mid-S phase, when replication of this gene has been completed (Iqbal et al., 1984) and transcription has returned to the basal level (Plumb et al., 1983; L. L. Baumbach et al., submitted for publication). Although differences detected in the repeat lengths could also reflect variations in the extent of digestion (Annunziato & Seale, 1982), it appears to be improbable considering the structural transitions observed with the 5′-histone H4 gene probe.

One possible explanation for this structural alteration in a cell cycle dependent human histone gene could be the general shortening that takes place in the linker region of newly replicated chromatin (D'Anna et al., 1982; Annunziato & Seale, 1982). These variations also occur when either DNA or protein synthesis is inhibited (D'Anna & Prentice, 1983; D'Anna & Tobey, 1984; Leffak, 1983; Poccia et al., 1984). However, the absence of such a change in the nucleosome structure of the β-globin gene following its replication does not support this explanation for the shortening of the nucleosomal spacing.

Another possible explanation for cell cycle dependent rearrangements in the chromatin structure of the H4 histone gene is that there is a functional relationship with the rate of transcription of the gene. This relationship is consistent with the temporal sequence of modifications in chromatin structure and histone gene transcription. The absence of observed changes in the chromatin structure of the β-globin gene, which is not transcribed in HeLa cells, supports such reasoning. Additionally, chromatin structure is not modified following inhibition of DNA replication and protein synthesis under the conditions we used (1-h treatment), although it is also possible that the short treatment only affects a small fraction of the replicating pF0108A extended region of the histone H4 gene. However, the random error does not seem to increase under the hydroxyurea and cycloheximide treatments, for which we can assume that there is not an increase in the heterogeneity of the chromatin fractions. These data provide further support for a relationship between histone gene transcription and chromatin structure since transcription of the histone genes is not blocked when HeLa cells are treated with DNA and protein synthesis inhibitors.

There appears to be a sequential series of modifications in the chromatin architecture of the human H4 histone gene that parallel changes in the extent to which the gene is transcribed during the cell cycle. When transcription is elevated early during S phase, there are changes in the chromatin structure of the 5′-flanking sequences containing promoter elements and sequences that modulate levels of transcription of this gene (Sierra et al., 1983; Shiels et al., 1985; Kroeger et al., unpublished results). Sequences in the 5′ region of the gene exhibit increased sensitivity to S1 nuclease (Chrysogelos et al., 1985), and a general disruption of the nucleosomal organization appears to occur both in the 5′-flanking sequences and in the mRNA coding region. This disruption of nucleosome organization we observe in the 5′-flanking and mRNA coding regions of the H4 gene in early S phase (Figure 5A) seems to be a general feature of active genes with a relatively high rate of transcription (Wu et al., 1979; Levy & Noll, 1981; Lohr, 1983, 1984). By mid-S phase when transcription of the H4 histone gene has been greatly reduced, there is evidence for a remodeling of histone gene chromatin structure. This is reflected by a broadening of the DNase I hypersensitive site in the 5′ promoter, a restoration of nucleosome organization,

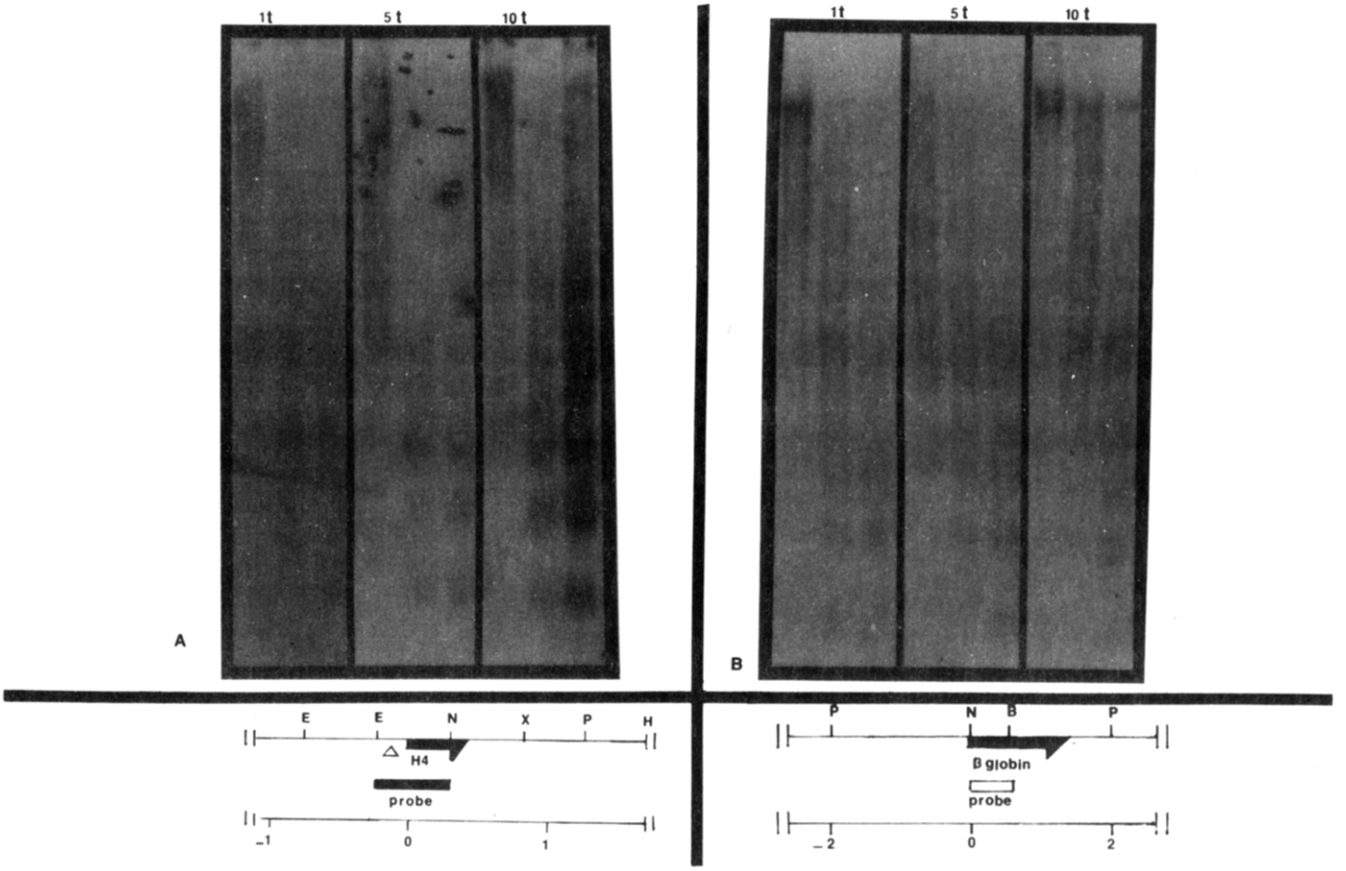

FIGURE 5: (A) Nucleosomal pattern of the *Nco*I–*Eco*RI segment of the H4 histone gene. This segment comprises 70% of the coding region, as well as the 5′-flanking sequences of the gene containing the DNase I sensitive site (indicated by a white triangle in the diagram). At 1 h after release from thymidine block (early S phase), no regular nucleosome ladder is generated by the micrococcal nuclease digestion of nuclei. A nucleosome ladder is evident in 5- and 10-h samples similarly treated. For each cell cycle time, the lanes represent 1, 2, and 5 min of nuclease digestion of the nuclei. E = *Eco*RI, N = *Nco*I, X = *Xba*I, and P = *Pst*I. (B) Nucleosomal pattern of the 5′ end of the protein coding region of the β-globin gene. A regular nucleosomal ladder is apparent for each cell cycle time analyzed. Lanes are as indicated for Figure 5A. The fragment used as probe is shown in the diagram in the lower portion of the figure. P = *Pst*I, N = *Nco*I, and B = *Bam*HI.

and a change in the nucleosome repeat length. Whether this type of nucleosomal organization is characteristic of genes that are expressed at a basal level but have periodic increases in transcription (such as during early S phase for the H4 histone gene we have been examining) remains to be determined. However, such changes in chromatin organization can offer the structural flexibility to fine-tune levels of transcription over short periods of time. At mitosis, with the general condensation of chromatin, there is again a reorganization of the chromatin structure of the H4 histone gene, resulting in a longer nucleosome repeat length. This lengthening of the nucleosome repeat may be related to the overall reduction in transcription that occurs during mitosis and a more ordered conformation of the chromatin that may involve the entire genome, since this value is found also in the total chromatin and β-globin gene. We cannot rule out the possibility of a cell cycle dependence in the repeat lengths of bulk chromatin as the progressive increase of this parameter seems to indicate (Table I). The complexity and heterogeneity of bulk chromatin make it impossible to analyze and correlate this possible cell cycle dependency with any specific event occurring in the cell cycle. Variations in nucleosome repeat length related to the cell synchronization procedure are also a possibility.

The distances that cell cycle dependent structural modifications extend upstream and downstream from the pF0108A H4 histone gene and the contribution of nucleotide sequences to levels of transcription remain to be determined. These results will provide insight into the functional limits of this gene and the relationship between the structural and functional properties of regulatory regions. The clustering of human histone genes (2–5 histone genes within 15 kb segments of DNA) raises the questions of whether there is overlap of the structural and functional limits of individual copies, and whether histone gene clusters contain broad domains. Within this context, it is interesting to note that in at least one cluster there are less than 750 bp between two histone genes, indicating that there is a high probability for structural and functional overlap.

The variations that have been reported in nucleosomal organization and chromatin structure for different genes (Smith et al., 1983; Weischet et al., 1983; Pederson et al., 1984; Wu & Simpson, 1985; Murray & Kennard, 1984) may be a reflection of differences in structure related to the mode or mechanism of expression. In the case of cell cycle dependent human histone genes, there is an elevated level of expression during early S phase that is transient, cyclic, and reversible. There are several other cell cycle regulated genes that show patterns of transcription similar to that of human histone genes. It will therefore be necessary to determine if these genes share similar changes in chromatin structure during the cell cycle.

Understanding the mechanisms that mediate the structural properties of chromatin and modifications in chromatin structure as a function of gene expression necessitates accounting for localized changes in nuclease sensitivity and long-range alterations in nucleosome spacing. Progress has been made in identification and characterization of proteins that influence nuclease sensitivity of several genes (Emerson & Felsenfeld, 1984; Wu, 1984). H1 histone has been im-

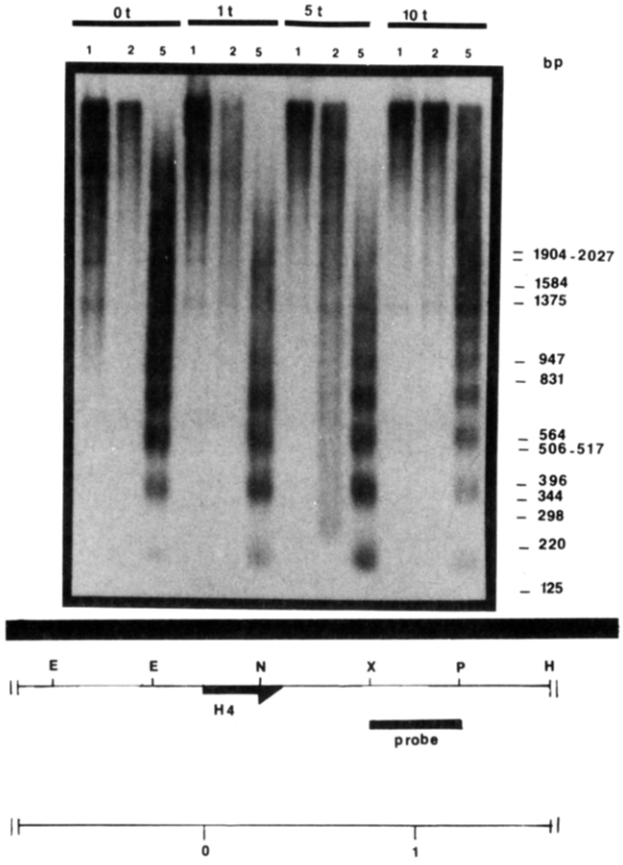

FIGURE 6: Nucleosomal pattern of the distal 3′-end segment of the histone H4 gene at 0, 1, 5, and 10 h after release from double thymidine block synchronization. E = *Eco*RI, N = *Nco*I, X = *Xba*I, P = *Pst*I, and H = *Hin*dIII. At each time point, nuclei were digested with 100 units/mL micrococcal nuclease for 1, 2, or 5 min (indicated at top of figure). The sizes of the marker fragments are indicated on the right side of the figure. The fragment used as hybridization probe is shown in the diagram at the bottom.

plicated as playing a role in nucleosome spacing (Weintraub, 1978; Savic et al., 1981; D'Anna & Prentice, 1983; Poccia et al., 1984); however, the mechanism for such long-range changes may involve propagation of a structural modification initiated at a single locus.

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