Condensation of chromatin into chromosomes preserves an open configuration but alters the DNase I hypersensitive cleavage sites of the transcribed gene

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ABSTRACT

DNase I was used to probe the molecular organization of the chicken ovalbumin (OV) gene and glyceraldehyde 3-phosphate dehydrogenase (GPD) gene in interphase nuclei and in metaphase chromosomes of cultured chicken lymphoblastoid cells (MSB-1 line). The OV gene was not transcribed in this cell line, whereas the GPD gene was constitutively expressed. The GPD gene was more sensitive to DNase I digestion than the OV gene in both interphase nuclei and metaphase chromosomes, as determined by Southern blotting and liquid hybridization techniques. In addition, we observed DNase I hypersensitive sites around the 5' region of the GPD gene. These hypersensitive sites were not always at the same locations between the interphase nuclei and metaphase chromosomes. Our results suggest that chromatin condensation and decondensation during cell cycle alters nuclease hypersensitive cleavage sites.

INTRODUCTION

Evidence from several sources suggests that transcriptionally or potentially active genes exist in an altered conformation in nuclear interphase chromatin. DNase I as well as other nucleases have been used as probes to preferentially digest a variety of transcriptionally active genes in chromatin. These include globin (1), ovalbumin (2,3), *Drosophila* heat shock genes (4), ribosomal genes (5), and a diversity of integrated viral genes (6-8). Transcriptional activity per se is apparently not required to maintain the "active" conformation, since genes that were once expressed but subsequently became inactive during normal development or in response to (or withdrawal from) acute environmental stimuli can still manifest the sensitive conformation (1,4,9). Moreover, a gene's degree of sensitivity to DNase I appears to be independent of the rate at which it is transcribed (10). These observations are consistent with the concept that sensitivity to DNase I may be a necessary, but not the only, condition for transcription (11).

During mitosis, replicated genetic material is segregated and condensed

into daughter chromosomes that exhibit very low level of transcriptional activity. The process of chromatin condensation is thought to involve repeated folding and coiling of fibers to form higher order structures (12,13). Clearly, it is possible that genes expressed in interphase nuclei might be placed into a structural conformation in condensed chromosomes that make them inaccessible to nucleases as well as RNA polymerases. However, the effect of chromatin condensation on the sensitivity of nucleases to the domains of expressed and repressed genes are not known. We report here the use of deoxyribonuclease (DNase I) as a probe to examine the nuclease sensitivity of two structural genes, glyceraldehyde-3-phosphate dehydrogenase (GPD) and ovalbumin (OV), within interphase nuclei and condensed metaphase chromosomes prepared from cultured chicken MSB-1 cells.

MATERIALS AND METHODS

Cell culture and isolation of nuclei and chromosomes

Chicken lymphoblastoid cell line MSB-1, which was established from a spleen tumor in a bird having Marek's disease, was used for this study. The karyotype of the cells has been shown to be normal, except for a single translocation (14). The cells were propagated at 41°C in McCoy's 5a medium supplemented with 10% fetal calf serum. The doubling time of cells cultured under these conditions is about 8 h. Synchronization of cells was achieved by a single mitotic block with Colcemid (0.06 µg/ml) for 6 h. A mitotic index of ~ 70% was commonly obtained.

Preparation of nuclei and chromosomes was the method described previously (15,16). Briefly, after Colcemid block, the cells were pelleted by centrifugation (2,000 rpm for 10 min. Sorvall GSA rotor) and resuspended in the cell culture medium at ~ 10⁶ cells/ml. Four volumes of twice-deionized water were added and the cell suspension was incubated at room temperature for 15 min. The cells were then pelleted by centrifugation and resuspended at 5x10⁷ cells/ml in chromosome isolation medium as described by Blumenthal et al. (17). After incubation in this chromosome isolation medium for 20 min, the cell suspension was forced through a 20-gauge needle six times to disrupt the cells and release the chromosomes. The lysed cell suspension (5 ml) was layered onto 20 ml of a 20% sucrose solution prepared in chromosome medium in a Corex tube. Nuclei were pelleted by centrifugation in a Sorvall HB 4 rotor at 2,000 rpm for 10 min. The supernatant fluid, which consisted of two layers (cell mixture and sucrose solution layers), was carefully removed one layer at a time and reassembled in another Corex tube. The chromosomes

were pelleted by centrifugation at 7,000 rpm for 20 min in the same rotor. Cross-contamination of chromosomes and nuclei in the fractions was monitored by staining the samples with ethidium bromide (20 $\mu\text{g}/\text{ml}$) and observing them in a Leitz fluorescence microscope. If necessary, the pelleted chromosomes or nuclei were resuspended in the chromosome isolation medium and subjected to another differential sedimentation procedure as described above to remove contaminating nuclei in chromosome sample or contaminating chromosomes in nuclei sample. Usually less than 5% cross-contamination was found in both chromosome preparations and nucleus preparations. This was calculated from the number of nuclei (or chromosomes) in an aliquot of the isolated chromosomes (or nuclei) using light microscopy, on the assumption that 28 chromosomes are equivalent to one nucleus. Chicken cells contain 28 light-microscopically identifiable macrochromosomes and some 40 microchromosomes that cannot be clearly seen by light microscopy.

RNA extraction, oligo(dT)-cellulose chromatography, and RNA-excess hybridization analyses

Total cytoplasmic RNA from chicken MSB-1 cells was isolated according to the procedure described previously (18). Polyadenylated RNA was obtained by passage of total cytoplasmic RNA through an oligo(dT)-cellulose column as described by Aviv and Leder (19).

The DNA probes were labeled with ^{32}P -dCTP by nick translation as described by Rigby et al. (20) with slight modifications (16). The probe for the ovalbumin gene was derived from a chicken genomic DNA clone $\lambda\text{C}_4\text{-ov5}$, which was recloned in the Bam HI site of pBR322 as described previously (20) (a gift from P. Chambon, Strasbourg, France). The probe for the chicken GPD gene (pGPD28, insert length of 1300 nucleotides) was constructed by inserting full-length double-stranded cDNA of muscle mRNA into the Pst site of plasmid pBR322 through a poly d(G)-poly d(C)-linker. A detailed description of construction of the recombinant DNA for GPD mRNA will be published elsewhere (22).

RNA-DNA hybridization reactions were performed in a reaction solution containing 3000 to 4000 cpm of ^{32}P -labeled DNA probe (10×10^6 cpm/ μg), 0.1 ~ 150 μg of RNA, 0.5 M NaCl, 0.01 M Hepes, and 2 mM EDTA, pH 7.0. The hybridization reactions (20 μl) were carried out in sealed capillary tubes and incubated at 68°C for various periods of time. Following incubation, the samples were expelled into 200 μl of the S1-nuclease digestion buffer that contained 0.4 M Na-acetate, 0.8 M NaCl, 5 mM ZnCl_2 , pH 4.5, and 15 μg of unlabeled Escherichia coli DNA. The samples were digested with 1000 units/

10 μ l of S1 nuclease (Miles Laboratory, Elkhart, Indiana) at 37°C for 30 min. The data from the hybridization experiments were expressed as CCl_3COOH precipitation followed by the percentage of hybridization vs. Rot (RNA concentration x time) (23).

Nuclease digestion, gel electrophoresis, and Southern blot

In preparation for micrococcal nuclease digestion, nuclei or chromosomes were resuspended in Hewish and Burgoyne buffer A (24) containing 0.5 mM CaCl_2 at $A_{260} = 25$ (10 μ l was diluted to 2 M NaCl for absorbance measurement). Digestion was carried out at 80 units of enzyme/ml of nuclei or 4.5 units/ml of chromosomes. Micrococcal nuclease was purchased from Worthington, Freehold, New Jersey. A limited DNase I digestion was performed under conditions in which nuclei or chromosomes were resuspended in a buffer containing 10 mM Tris-HCl, pH 7.5, 10 mM NaCl, and 3 mM MgCl_2 at $A_{260} = 25$ and digested with 2.5 μ g DNase I/ml of nuclei or 0.25 μ g/ml of chromosomes. In the experiments on more extensive digestion with DNase I, either 20 μ g DNase I per ml of nuclei or 2 μ g DNase I per ml of chromosomes was used. DNase I was purchased from Sigma, St. Louis, Missouri. All the enzyme reactions were carried out at 37°C for various periods of time from 2 min to 30 min and terminated by the addition of 0.2 M EDTA to a final concentration of 15 mM.

Following nuclease treatment, DNA was isolated, digested with restriction endonuclease, and separated by electrophoresis on agarose slab gel according to protocols of Bellard et al. (21). Southern blot (25) and analysis of differential nuclease sensitivities from autoradiographs were described in a previous communication (26). The relative intensities of autoradiographic bands were determined by densitometric scans (Quick Scan, Helena, Co., Beaumont, Texas).

DNA excess hybridization

DNA from high-level DNase I-digested chromosomes or nuclei samples was used as driver DNA in hybridization with radioactively labeled probes prepared from the inserts of recombinant DNA clones containing chicken ovalbumin (OV) and glyceraldehyde-3-phosphate dehydrogenase (GPD) genes. Before hybridization, the driver DNA was sonicated with a Branson cell disrupter using a microtip at a setting of 6 at 0 - 4°C. Sonication was 15 sec at a time with 2-min interval and total sonication time of 3 min. The sonication produces DNA with single-stranded length of approximately 600 nucleotides as analyzed by agarose (1.6%) gel electrophoresis under alkaline conditions (30 mM NaOH and 2 mM EDTA) with ^{32}P -end-labeled Hind III-digested SV40 DNA as molecular

weight markers. The procedure for DNA annealing was described previously (18).

RESULTS

Different abundance of the ovalbumin and the glyceraldehyde-3-phosphate dehydrogenase gene transcripts in the MSB-1 cells

Two different chicken gene probes were used in the present study, i.e. the ovalbumin (OV) and the glyceraldehyde-3-phosphate dehydrogenase (GPD) genes. The OV gene is normally expressed in the estrogen response oviduct while the GPD gene is constitutively expressed as a house-keeping glycolytic enzyme in all cells. The amount of these transcripts in total cytoplasmic poly(A)⁺ RNA from MSB-1 cells was quantitated by solution hybridization. Figure 1 presents results obtained from such hybridization experiments by Rot analysis using ³²P-labeled OV and GPD DNA probes. The GPD gene probe hybridized with the RNA to an expected 50% completion with Rot_{1/2} value of 5.0 mol. sec; whereas the OV gene probe failed to hybridize even at the Rot value of 80. These data indicated that the GPD gene is transcribed and GPD mRNA accumulated in the MSB-1 chicken cell culture, while the OV gene appears to be repressed. According to the method described by Bishop et al. (27), we estimate that the one MSB-1 cell contains approximately 600 copies of the GPD and <1 copy of the OV-gene transcripts.

Differential response of the ovalbumin gene and the GPD gene to the DNase I digestion in interphase nuclei and in metaphase chromosomes

The transcribing (or potentially transcribing) chromatin in the eukaryotic genome is preferentially sensitive to digestion by deoxyribonucleases (1-11). The degree of nuclease sensitivity of genes in chromatin can be analyzed by either liquid hybridization or Southern blot hybridization. The solution hybridization assay measures the rate at which a given region of a gene is digested to nonhybridizable fragments, while the blot hybridization assay measures the probability of introducing the first nuclease cut into a given fragment, which may, or may not, be homogeneously more accessible to the nuclease (20). We first used the solution hybridization technique (Cot analysis) to determine the sensitivity of the OV and GPD genes to extensive nuclease digestion. Nuclei and chromosomes prepared from synchronized chicken MSB-1 cells were digested with DNase I to render 23% and 25% acid solubility, respectively. The DNA were extracted, sheared by sonication, and used as drivers for hybridization with tracer ³²P-labeled OV gene and GPD gene probes. The results of these experiments are shown in

Fig. 1. Hybridization analysis of the RNA transcripts in the chicken MSB-1 cells. The DNA inserts containing the GPD gene and the OV gene in the corresponding recombinant DNA plasmids were nick translated with ^{32}P -dCTP and used as the hybridization probes. Hybridization was performed against a large excess of poly(A)-containing RNA, which was isolated from the MSB-1 cells as described (14,15). (O-O), RNA-DNA GPD hybridization; (●-●), RNA-DNA OV hybridization.

Figure 2. These two gene probes hybridized at a rate similar to the sonicated MSB-1 nuclear DNA with $\text{Cot}_{1/2}$ of 1500, indicating that the MSB-1 cells contain a similar copy number of these genes per genome. In the nuclease-digested nuclei (Fig. 2B) or chromosome (Fig. 2C), the hybridization curves showed that the GPD gene probe hybridized at a slower rate ($\text{Cot}_{1/2} = 6000$) than did the OV gene probe ($\text{Cot}_{1/2} = 2000$). These results suggest that under extensive nuclease digestion, the constitutively expressed GPD gene in both interphase nuclei and metaphase chromosomes was 3 to 4 times more sensitive to enzyme treatment than the transcriptionally arrested OV gene. Stalder et al. (28) reported that in *Physarum polycephalum*, chromatin containing ribosomal RNA genes in mitotic cells and in G_2 cells are equally sensitive to DNase I-digestion.

We then utilized the Southern blotting hybridization technique to analyze the differential sensitivity of the OV gene and the GPD gene to nuclease digestion. The nuclei and chromosome samples were mildly digested with DNase I, and the DNA was isolated from the digested samples. The DNA was subsequently cleaved with the restriction enzyme *EcoRI*, separated by gel electrophoresis, transferred to a nitrocellulose filter by the Southern blotting procedure (25), and hybridized with ^{32}P -nick-translated DNA probes containing both GPD and OV gene sequences.

An autoradiograph of a limited nuclease digest of MSB-1 nuclear DNA is shown in Figure 3A. The undigested control sample (lane 1) revealed four

Fig. 2. Liquid hybridization analysis of nuclease sensitivity of GPD and OV genes in interphase nuclei and metaphase chromosomes. DNA isolated from DNase I-undigested nuclei (A), digested nuclei (B), or chromosomes (C) were sonicated and used as drivers to hybridize with ^{32}P -labeled probes of OV gene (O-O) or GPD genes (Δ - Δ). Hybridization conditions are described in Materials and Methods.

bands that corresponded to *Eco*RI fragments 15 kb, 14 kb, 9.2 kb and 2.35 kb in length. The 15-kb fragment is complementary to pGPD28 DNA and is referred to as GPD 15-kb. The other fragments are complementary to the OV gene probe. In the samples digested with nuclease for various periods of time, the intensity of the band corresponding to GPD 15-kb disappeared faster than did those of the OV 14-kb and OV 9.2-kb fragments (Fig. 3A, lanes 2-5). These autoradiographs were scanned with a densitometer and the area under each autoradiographic peak was integrated (Fig. 3B). The relative content of each fragment was normalized to the undigested control samples. The analysis showed that the GPD 15-kb was approximately 2.5-fold more sensitive than the OV 14-kb to the nuclease digestion. We chose OV 14-kb for comparison because the GPD 15-kb is only 1.07-fold longer than the OV 14-kb fragment.

The nuclease sensitivities of GPD and OV genes were then examined in condensed metaphase chromosomes. Chromosomes prepared from synchronized MSB-1 cells were mildly digested with DNase I and analyzed as described above. The results of this experiment, as shown in Figure 4A, revealed that the GPD 15-kb fragment was more susceptible to DNase I than any of the OV fragments. Differential sensitivity analyses as described above demonstrated that the

Fig. 3. Kinetics of DNase I sensitivity for the GPD gene and the OV gene in chicken MSB-1 cell nuclei. DNA from untreated nuclei and nuclei treated with DNase I for various periods of time were digested with the restriction enzyme *EcoRI*, separated by 0.7% agarose gel, blotted, and hybridized to nick-translated clone DNAs containing GPD and OV genes. A, Autoradiogram. Lane 1, control, no DNase I was used; lanes 2-5, correspond to nuclease digestion time of 3, 5, 10, and 30 minutes, respectively. The GPD gene probe hybridized only to the 15-kb and 8.6-kb fragments; the OV gene probe hybridized with the 14-kb, 9.2-kb, and 2.35-kb fragments. The 1.75-kb fragment was run off the gel. Note that GPD 8.6-kb fragment is not present in the control sample. B, Densitometric scans of the autoradiogram. Only the upper part of the autoradiogram was scanned. Scans 1 to 5 correspond to lanes 1 to 5 shown in A. Note the peaks for the GPD 15-kb fragments disappear at a faster rate than those for the OV 14-kb gene fragment from scans 1 to 5.

GPD 15-kb fragment was at least threefold more sensitive to nuclease digestion than the OV 14-kb species. These results indicate that even in highly condensed metaphase chromosomes, a constitutively expressed gene maintains its preferential sensitivity to nuclease.

DNase I hypersensitivity cleavage site in the interphase nuclei and metaphase chromosomes

As shown in Figure 3A, a restricted fragment with 8.6 kb in length is

Fig. 4. DNase I sensitivity of glyceraldehyde 3' phosphate dehydrogenase and ovalbumin genes in chromosomes isolated from synchronized chicken MSB-1 cells. A, Autoradiogram. Lane 1, nuclease-undigested control sample. Lanes 2-5, DNase I-digested samples with digestion time corresponding to 3, 5, 10, and 30 min. Note that 6.3-kb fragment was not present in the control sample. B, densitometric scans of the autoradiograph. Note that the peak for the GPD 15-kb fragment disappears at a faster rate than that for the OV 14-kb fragment as the digestion time increases.

present in the DNase I-digested nuclei samples (lanes 2-5) but not in the undigested control (lane 1). This fragment hybridized specifically with pGPD28 DNA but not with the OV gene probes (data not shown). This observation is consistent with previous reports that higher order chromatin structures may form nuclease specific double-stranded DNA cuts (termed a hypersensitive site) around actively transcribed genes (29-33). It is important to note that the 8.6-kb fragment is not present in the nuclease-digested chromosome samples (Fig. 4A, lanes 2-5). Instead, another new fragment of 6.3 kb that is complementary to pGPD28 emerged from nuclease-digested chromosomes. Interestingly, the 6.3-kb fragment was not present in the DNase I-digested nuclei samples (Fig. 3A).

We utilized the restriction enzyme Hind III to help locate the hypersensitive sites (Fig. 5). In the undigested nuclei sample, the pGPD28 gene probe hybridized with three restriction fragments of 8.0 kb, 1.6 kb, and 0.4 kb. The 8.0-kb fragment represents the 5' terminus and flanking region of the GPD gene, since it only hybridized with the 5' sequence of the pGPD28 insert (unpublished data). In the DNase I-digested interphase nuclei (Fig. 5A), at least three additional fragments 5.4 kb, 4.0 kb and 2.2 kb long were present; they represent three hypersensitive sites within the 8.0-kb fragment. The 5.4-kb and 4.0-kb fragments, but not the 2.2-kb fragment, were also present in the nuclease-digested chromosome samples (Fig. 5B). The absence of the 2.2-kb fragment in the nuclease-digested

Fig. 5. Kinetics of DNase I sensitivity for the GPD gene in chicken MSB-1 cell nuclei, metaphase chromosomes, and purified DNA. Nuclei and chromosomes were digested with DNase I for various periods of time and analyzed as described in Materials and Methods. A, nuclei. Lane 1, undigested control; lanes 2 to 5, samples were digested with DNase I for 2, 4, 8, and 20 min, respectively. B, chromosomes. Lanes 1 and 2, samples were digested for 4 and 12 min, respectively. C, DNase I digested purified DNA. Lanes 1 to 3, DNA isolated from nuclei was digested with DNase I (0.01 $\mu\text{g/ml}$) for 2, 4, and 8 min, respectively; lane 4-7, DNA isolated from chromosomes were digested with DNase I for 2, 4, 8 and 10 min, respectively.

chromosome samples was not likely due to underdigestion of chromosomes by DNase I, since the band for the 8.0-kb. fragment was extensively digested. These results, together with those shown in Fig. 3A and 4A, indicate that condensation of chromatin into chromosomes altered the locations of nuclease hypersensitive cleavage sites. We have reason to believe that the above-mentioned hypersensitive cleavage is due to chromosome structure rather than nucleotide specificity of the nuclease. This conclusion is based on the result of a control experiment that showed the nuclease does not produce strong specific cleavage in the purified chicken nuclear or chromosomal DNA (Fig. 5C).

Transcribed gene and non-transcribed gene maintain the same nucleosome repeat length between interphase nuclei and metaphase chromosomes

We next determined whether the change of nuclease hypersensitive sites between interphase nuclei and metaphase chromosomes was due to differences in nucleosome repeat lengths between interphase nuclei and metaphase chromosomes. Nuclei and chromosome samples were digested with micrococcal nuclease. DNA was extracted from the digested samples, separated by agarose gel electrophoresis, transferred to a nitrocellulose filter by the Southern procedure, and hybridized with ^{32}P -labeled probe for the transcribed (GPD) and nontranscribed (OV) gene sequences. Figure 6A shows an ethidium bromide staining pattern of a gel containing nucleosomal DNA of nuclei and metaphase chromosomes. The overall nucleosomal DNA repeats between these two samples are the same. Using restriction fragments of known length (i.e. Hind III-digested PM2 phage DNA and Hind III-digested SV40 DNA) as molecular weight markers, the repeat length of nucleosomal DNA in MSB-1 cell nuclei and chromosomes were calculated to be 180 ± 5 base pairs. Autoradiograms of the Southern blots using OV gene and GPD gene probes in Figs. 6B and 6C, respectively, showed that the nucleosome lengths of these gene sequences were not different between interphase chromatin and metaphase chromosomes. These results indicate that the condensation of chromatin during the cell cycle does not change the nucleosomal repeat length of transcribing and nontranscribing genes.

DISCUSSION

In spite of a number of studies, the molecular basis underlying the acquisition of DNase I sensitivity in "active chromatin" is still not well understood. Weisbrod et al. (35) demonstrated that HMG proteins 14 and 17, perhaps together with other chromosomal factors, are probably responsible for the increased susceptibility of DNA to nucleases. HMG proteins 14

Fig. 6. Analyses of repeat lengths of nucleosomes containing OV gene and GPD gene in interphase nuclei and metaphase chromosomes. Nuclei and metaphase chromosomes were digested with micrococcal nuclease for various periods of time. DNA were extracted and separated by 2% agarose gel electrophoresis, transferred to nitrocellulose filter and hybridized with ^{32}P -labeled gene probes. A, Ethidium bromide staining pattern. Lane 1, restriction fragments of Hind III-digested SV40 DNA (from the top 1768, 1169, 1101, 526, 447 bp); lanes 2-5, nuclei samples digested with micrococcal nuclease for 3, 6, 9 and 12 min, respectively; lanes 6-9, chromosome samples digested with micrococcal nuclease for 2, 4, 9, and 20 min, respectively; lane 10, molecular weight markers (Hind III digested PM2 DNA, from the top: 5151, 1969, 833, 424, 400, 227 bp). B, Southern blot of nucleosomal DNA hybridized with the OV gene probe. C, Southern blot of the nucleosomal DNA hybridized with the GPD gene probe. Lanes 1 through 8 in B, and C correspond to lanes 2 through 9 described in A. DNA samples in B and C are from two independent experiments.

and 17 can restore DNase I sensitivity to purified nucleosomal particles depleted of HMG. These results have been interpreted to suggest that "active" genes reside within chromatin subunits of altered conformation. How the HMG proteins might interact with nucleosomes of the potentially transcribed genes in metaphase chromosomes remains to be investigated. It is also noteworthy that although the transcriptional activity of metaphase chromosomes is very low, a significant amount of RNA polymerase B (or II) has been found to associate with the chromosomes (Garioglio, P., Bellard, M. and Chambon, P., Strasbourg, France, personal commu.). It also remains to be investigated how RNA polymerase molecules (or factors for gene transcrip-

tion) interact with the structural genes in condensed chromosomes and the role that these molecules play in conferring the structural genes in metaphase chromosomes to be preferentially sensitive to DNase I digestion.

In the S phase of the cell cycle, Weintraub (36) showed that the actively transcribed genes are assembled into structures characteristic of actively transcribed nucleosomes (restoring DNase I sensitivity) with 20 nucleosomes of the replication fork (about 3 minutes after the onset of DNA replication). We presented evidence here that the open configuration of chromatin containing a transcribing gene, GPD, was maintained during metaphase stage. These findings taken together suggest that the transcribable genes remain in an open configuration throughout the cell cycle.

In this study, we have observed that DNase I introduced a specific double-stranded cut into the region associated with the GPD gene domain. Specific cutting by DNase I was first observed by Wu et al. (29) in several Drosophila genes and subsequently by other investigators (30-34) in other systems. Most of the DNase I hypersensitive sites have been assigned to the 5' sides of the structural genes, where a nucleosome-free region may be located (34,37-39). In a general agreement, the location of the DNase I hypersensitive sites around the GPD gene region appears to be near the 5' terminus. However, the most significant observation from our work is that some of these specific cleavage sites are cell cycle-specific. These observations are consistent with the concept that during chromatin condensation and decondensation in the cell cycle, the DNase hypersensitive regions may play a role in modulating the molecular architecture of metaphase chromosomes.

Finally, we favor the notion that detection of the nuclease hypersensitive cleavage in chromatin depends upon the structure of the chromatin domain, the methods of analysis, and the specific probes employed. We have found a DNase I hypersensitive site in the MSB-1 chromatin containing another house-keeping gene, the thymidine kinase gene (a gift by M. Wigler, Cold Spring Harbor Lab.). However, the hypersensitive site is not relocated during chromatin condensation. The failure to detect nuclease hypersensitive cleavage in the ovalbumin gene region reported and in other studies (26,32) may also be consistent with this notion.

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