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Genetics

Deoxyribonuclease I sensitivity of plasmid genomes in teratocarcinoma-derived stem and differentiated cells

(DNase I/chromatin/herpes simplex virus/thymidine kinase gene/differentiation)

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ABSTRACT **The DNase I (EC 3.1.21.1) sensitivities of the simian virus 40 (SV40) genome, the pBR322 genome, and the herpes simplex virus type 1 thymidine kinase (HSV-1 *tk*) gene have been compared in teratocarcinoma-derived stem (12-1) and differentiated (12-1a) cell lines established by transfection of thymidine kinase (ATP:thymidine 5′-phosphotransferase, EC 2.7.1.21)-deficient F9 cells with DNA from a tripartite plasmid genome consisting of the pBR322 genome, the SV40 genome, and the HSV-1 *tk* gene. HSV-1 *tk* is present in both stem and differentiated cells; SV40 early proteins are present in differentiated cells but not in stem cells; the pBR322 genome is not expressed in either cell type. The SV40 and pBR322 genomes are more sensitive to DNase I digestion in stem cells than in differentiated cells, reflecting the DNase I-hypersensitivity of total stem-cell chromatin. The HSV-1 *tk* gene is the least sensitive to DNase I digestion in both cell types.**

The eukaryotic genome is organized in nucleosomes, chromatin subunits that consist of DNA complexed with histones and nonhistone chromosomal proteins (for review, see refs. 1 and 2). As it is probable that differences in chromatin organization are involved in regulation of gene expression, it is essential to devise experiments that can relate differences in structure at the chromatin level to differential gene expression.

Nuclease digestion studies have produced evidence that active and inactive chromatin has a repeating subunit structure and have provided a means to dissect the internal organization of the nucleosome (for review, see ref. 3).

DNase I probing of chromatin structure has provided evidence that active chromatin has an altered more accessible ("open") configuration than inactive chromatin; that is, actively transcribed genes are preferentially digested by DNase I. Thus, the globin gene and not the ovalbumin gene is digested in avian erythrocytes (4) whereas the ovalbumin gene and not the globin gene is preferentially digested in chicken oviduct (5).

Preferential sensitivity to DNase I has been detected for other active genes, such as integrated viral genomes (6–11) and the heat-shock loci in *Drosophila* (12). In addition, it has been observed that there are specific sites in chromatin that are hypersensitive to cleavage by DNase I (13–16). These sites can be precisely mapped relative to the restriction map of unique genes (17–21). As such hypersensitive sites are usually not found in the coding regions of genes (18), the structural basis for these sites may not be the same as the structural basis for preferential degradation of active genes as initially described by Weintraub and Groudine (4). Thus, nucleases have proved to be valuable tools in the investigation of chromatin structure, and we have initiated studies of chromatin structure in teratocarcinoma-derived stem and differentiated cells. We have compared the DNase I sensitivity of the pBR322 genome, that of the simian virus 40 (SV40) genome, and that of the herpes simplex virus type 1 thymidine kinase (HSV-1 *tk*) gene by using the stem cell 12-1 and its differentiated daughter cell 12-1a, which contain one copy of the pBR322/HSV-1 *tk*/SV40 recombinant plasmid (pC6) integrated through a site on the pBR322 genome (22). We found that the pBR322 genome, which is not expressed in either cell type, was more sensitive to DNase I digestion in stem than in differentiated cells; that the SV40 genome, which is not expressed at the protein level in stem cells, was also more sensitive to DNase I digestion in stem than in differentiated cells; and that the HSV-1 *tk* gene, which is constitutively expressed in stem and differentiated cells, was the least sensitive to DNase I digestion in both cell types.

MATERIALS AND METHODS

Cells. 12-1 stem cells were isolated after transfection of F9 thymidine kinase-deficient (TK^-) cells with pC6 and 12-1a differentiated cells were derived by cloning of a retinoic acid-treated 12-1 culture (22). The 12-1 and 12-1a cells have been characterized as stem and differentiated cells (23). PCC4 aza (24), OTT6050A F1 Brdu (25) $F9TK^-$ (26), and F9 (27) cells are teratocarcinoma-derived stem cell lines that were used in some studies; the F9ACcl9 cell line is a differentiated parietal endoderm line (23) derived by cloning of retinoic acid-treated F9 cells. All cells were maintained in RPMI 1640 medium/10% fetal bovine serum. Growth medium for 12-1 and 12-1a cells contained hypoxanthine/aminopterin/thymidine/glycine as described (22). Growth medium for OTT6050A F1 Brdu and $F9TK^-$ cells contained 5-bromodeoxyuridine (30 μg/ml), and medium for PCC4 aza cells contained 8-azaguanine (15 μg/ml).

Isolation of Nuclei. All manipulations after collection of cells were at 4°C. Cells were scraped from glass bottles and washed twice in phosphate-buffered saline and once in reticulocyte standard (RS) buffer (0.01 M Tris·HCl, pH 7.4/0.01 M NaCl/3 mM $MgCl_2$), and nuclei were isolated by suspension in RS buffer/0.5% Nonidet P-40 essentially as described by Weintraub and Groudine (4). Nuclei were washed twice with RS buffer and then suspended in it at 1 mg/ml (OD_{260}, 20) for DNase I digestion studies.

Isolation of Chromatin. Native chromatin was isolated from 12-1 and 12-1a cells by published procedures (28); briefly, nuclei were isolated by homogenization in hypotonic buffer, and 0.25 vol of 1 M sucrose was added and mixed by homogeniza-

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Abbreviations: kb, kilobase(s); TK, thymidine kinase; HSV, herpes simplex virus; SV40, simian virus 40; HSV-1 *tk*, herpes simplex virus type 1 thymidine kinase gene; pC6, pBR322/HSV-1 *tk*/SV40 recombinant plasmid; TK^-, thymidine kinase deficient.

tion. The homogenate was centrifuged, and the nuclear pellet was suspended by homogenization in 2.2 M sucrose and centrifuged at 30,000 × *g* for 1 hr at 4°C. Nuclei were suspended in 1% Triton X-100 and centrifuged at 12,000 × *g* for 10 min at 4°C; the pellet was suspended in 0.14 M NaCl and centrifuged at 12,000 × *g* for 10 min to obtain chromatin pellets. Chromatin pellets were suspended in RS buffer at 1 mg/ml (OD_{260}, 20) for DNase I digestion studies.

Nuclease Digestion. Three types of DNase I digestion experiments were performed.

Method 1. Initial DNase I digestions were carried out as described by Weintraub and Groudine (4) to determine the extent of digestion of nuclear DNA of stem and differentiated cells at various times after addition of DNase I. Nuclei were washed several times in RS buffer and digested at a DNA concentration of 1 mg/ml at 37°C with pancreatic DNase I (Worthington) (10 μg/ml) for various periods of time (usually 0, 2, 4, 8, 16, and 32 min after addition of DNase I). Samples without DNase I were taken at 0 and 32 min. The percentage of DNA remaining was determined by sedimenting the nuclei at low speed and measuring the release of material absorbing at 260 nm. Nuclei were then suspended in lysis buffer (0.01 M Tris/0.01 EDTA/0.6% $NaDodSO_4$, pH 7.4) containing proteinase K at 50 μg/ml (Boehringer Mannheim) and incubated at 37°C for 10–15 hr before DNA extraction.

Method 2. For mixing experiments, 12-1 and 12-1a cells were grown in medium containing [*methyl*-^{3}H]thymidine (5 μCi/ml, 1 Ci = 3.7×10^{10} becquerels; New England Nuclear) to label nuclear DNA. Labeled and unlabeled 12-1 and 12-1a cells were scraped from the bottles and counted. Equal numbers of labeled 12-1 and unlabeled 12-1a (or unlabeled 12-1 and labeled 12-1a) cells were mixed, and nuclei were isolated as above. In the same experiment, nuclei of 12-1 alone and 12-1a alone were prepared. The four nuclei samples were then treated with DNase I as described above. Percentage of DNA remaining at each time after DNase I treatment was calculated by determination of perchloric acid-insoluble labeled DNA remaining (4). Nuclear pellets were lysed in buffer containing proteinase K as in method 1.

Method 3. By the above two methods, we determined the degree of DNase I degradation of DNA in stem and differentiated nuclei (Table 1). Thus, in subsequent experiments, DNase I-treated samples (nuclei or chromatin) were plunged immediately into lysis buffer/proteinase K without prior sedimentation of nuclear pellets.

Table 1. Percent DNA remaining after DNase I digestion

Digestion time, min	12-1a	12-1	12-1a mixed*	12-1 mixed*
10 μg/mg of DNase I/mg of DNA				
2	88	85		
4	85	73		
8	80	66	60	60
16	73	59		
32	60	50	50	45
64	50	40		
1 μg/mg of DNase I/mg of DNA				
18	NT	90		

Percent DNA remaining was determined by sedimenting nuclei at low speed and measuring release of material absorbing at 260 nm. NT, not tested.

* [*methyl*-^{3}H]Thymidine-labeled 12-1a (or 12-1) cells were mixed with an equal number of unlabeled 12-1 (or 12-1a) cells; nuclei were isolated and DNase I treated; percent labeled 12-1a (or 12-1) DNA remaining was determined by precipitation with cold 7% perchloric acid and assay of acid-insoluble ^{3}H-labeled material.

In addition, 12-1 nuclei were treated with DNase I at low concentrations (0.01, 0.1, and 1 μg of DNase I/mg of DNA) and processed as in method 1.

Micrococcal nuclease (Worthington) digestion (10 units/mg of DNA) was carried out by method 3.

DNA was isolated from all samples as described (22).

To test DNase I sensitivity of plasmid genomes within naked cellular DNA, purified high molecular weight DNA from 12-1 and 12-1a cells was dissolved in RS buffer, and DNase I digestion (0.1 μg of DNase I/mg of DNA) was carried out (0, 2, 8, and 32 min) by method 3. Digested DNA samples were purified by proteinase K (Boehringer Mannheim) treatment followed by phenol, phenol/chloroform, and chloroform extractions and precipitated in ethanol; concentrations were determined spectrophotometrically.

*Bam*HI (Boehringer Mannheim) restriction enzyme digestion of purified DNA was carried out as prescribed by the manufacturer.

Gel Electrophoresis and Southern Transfer. Agarose gel (0.7% or 1.0%) electrophoresis was carried out in 40 mM Tris/5.0 mM NaOAc/2.0 mM EDTA, pH 8.0. *Hae* III-digested φX174 (0.8 μg per lane) or *Hin*dIII-digested λDNA (0.75 μg per lane) (or both) (Bethesda Research Laboratories, Rockville, MD) M_r standards were included on every gel. DNase I- or DNase I/*Bam*HI-digested DNA (10 μg per lane) samples were subjected to electrophoresis in a horizontal agarose (Sigma) slab gel. Gels were stained for 1 hr with ethidium bromide (1 μg/ml) and photographed under UV light. For gels to be processed by the Southern transfer method, a reconstruction lane was included that contained 10 pg of *Bam*HI-digested pC6 DNA. Transfer of DNA from gel to nitrocellulose sheet (Schleicher & Schuell, Ba 85) was performed essentially as described by Southern (29).

Preparation of Labeled Probe DNA. Covalently closed circular SV40 DNA and pBR322 DNA were purchased from Bethesda Research Laboratories. The HSV-1 *tk Bam*HI fragment was prepared by *Bam*HI digestion of pC6 DNA, electrophoresis in an agarose gel, and electroelution and purification of the 3.4-kilobase (kb) fragment from agarose. pBR322 and SV40 DNAs were at times prepared this way also.

^{32}P-Labeled SV40, pBR322, and HSV-1 *tk* DNAs with specific activities of $0.7–5 \times 10^8$ cpm/μg were prepared by the nick-translation procedure (30). DNA polymerase I was purchased from Boehringer Mannheim; [α-^{32}P]dNTPs were from Amersham.

Hybridization. DNA on nitrocellulose sheets was hybridized to ^{32}P-labeled probe DNA (SV40, pBR322, or HSV-1 *tk* DNA) in a hybridization solution containing 50% formamide (22). When ^{32}P-labeled HSV-1 *tk* DNA was used as the probe, unlabeled sonicated denatured *Escherichia coli* DNA (5 μg) and unlabeled denatured pBR322 DNA (2 μg) were added to the hybridization solution. When ^{32}P-labeled pBR322 DNA was used as the probe, unlabeled sonicated denatured *E. coli* DNA (5 μg) was added to the hybridization solution. After hybridization, the filters were washed, air dried, and exposed to XRP-5 film for various periods.

RESULTS

Nuclease Sensitivity of Total DNA in Stem and Differentiated Cells. When DNase I digestion was carried out using 10 μg of DNase I/mg of DNA by method 1 and the purified DNAs were subjected to electrophoresis in an agarose gel, a marked difference in sensitivity of stem and differentiated nuclear DNA was observed (Fig. 1 *A* and *B*; Table 1). The DNA of 12-1 nuclei was more rapidly digested (Table 1), and the average molecular weight of the DNA fragments produced was much lower (Fig.

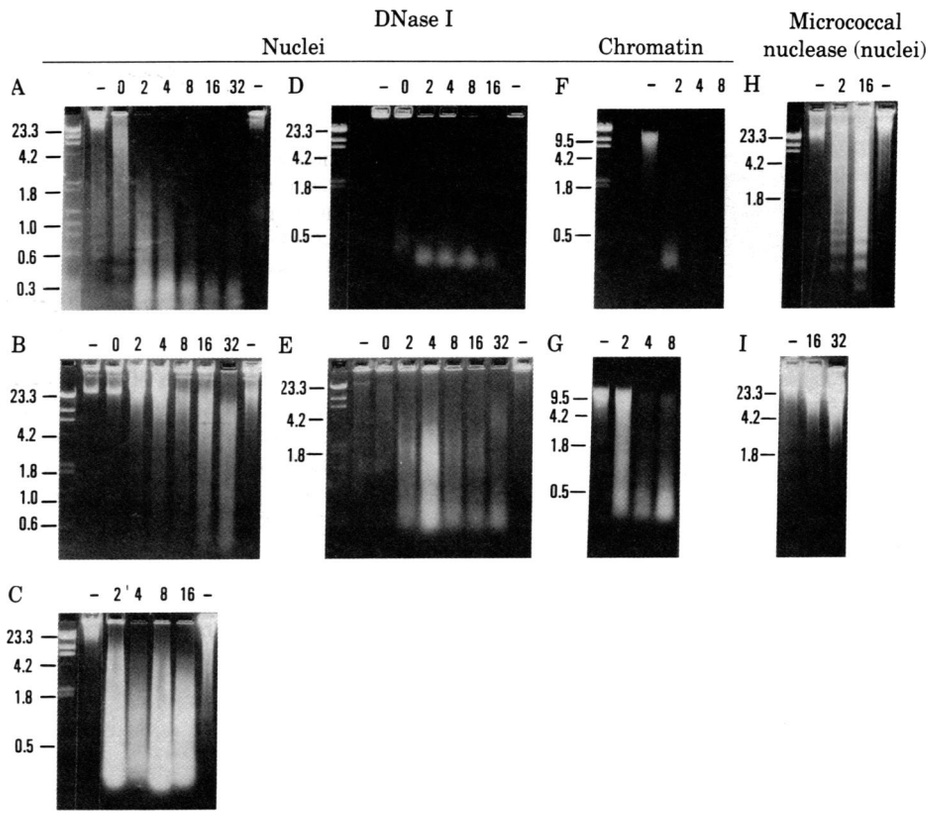

FIG. 1. Nuclease digestion of stem and differentiated cell nuclei and chromatin. DNase I digestions were performed by method 1, using 10 μg of DNase I/mg of DNA. Micrococcal nuclease digestions were performed by method 3, using 10 units of micrococcal nuclease/mg of DNA. DNA was isolated from each sample and subjected to electrophoresis (10 μg per lane) on 1.0% agarose gels for various periods of time (7–15 hr). Numbers across the top of each figure indicate the time (min) of digestion with nuclease and those on the left indicate sizes of fragments (kb pairs); minus signs above the left lanes indicate nuclei or chromatin samples incubated at 37°C for 2 min without nuclease; minus signs above the rightmost lanes in some figures indicate nuclei samples incubated for 32 min at 37°C without nuclease. (*A* and *D*) 12-1 and F9 stem cell nuclei digested with DNase I. (*B* and *E*) 12-1a and F9ACc19 differentiated cell nuclei digested with DNase I. (*C*) Equal numbers of 12-1 and 12-1a cells mixed before isolation of nuclei and then digested with DNase I. (*F* and *G*) 12-1 and 12-1a chromatin digested with DNase I; the 12-1 4- and 8-min lanes (*F*) contained only 4 μg of DNA. (*H* and *I*) 12-1 and 12-1a nuclei digested with micrococcal nuclease.

1*A*) than those from 12-1a cells (Fig. 1*B*). This result is not due to endogenous nuclease activity in stem cell nuclei as DNA from 12-1 nuclei incubated at 37°C without DNase I for 32 min (the duration of most experiments) is not degraded (Fig. 1*A*, rightmost lane). In experiments in which equal numbers of 12-1 and 12-1a cells were mixed and the nuclei were isolated and DNase I treated together, we found that digestion of DNA in 12-1a nuclei is accelerated (Table 1), and the electrophoretic pattern of DNA isolated from mixed digestion displays a size distribution intermediate (Fig. 1*C*) between the patterns of 12-1 alone (Fig. 1*A*) and 12-1a alone (Fig. 1*B*).

To determine whether hypersensitivity to DNase I is a general property of chromatin in murine teratocarcinoma stem cells, we performed DNase I digestion experiments with three other stem cell lines: (*i*) F9, the stem cell parent of 12-1; (*ii*) PCC4 aza; and (*iii*) OTT6050A F1 Brdu. We found for each a sensitivity pattern similar to that of 12-1 nuclei (see Fig. 1*D* for an example). To determine whether hyposensitivity to DNase I is characteristic of differentiated cells, we subjected F9ACcl9 (a differentiated clone derived from F9 that does not contain plasmid genomes) nuclei to DNase I digestion under the same conditions (10 μg of DNase I/mg of DNA, 0–32 min) and observed the electrophoretic pattern produced by the purified DNA at different time points. The DNase I-digested F9ACcl9 nuclear DNA (Fig. 1*E*) was of a higher average molecular weight than DNase I-digested 12-1 DNA (Fig. 1*A*) or F9 DNA (Fig. 1*D*) but was of a lower average molecular weight than similarly derived 12-1a DNA (Fig. 1*B*). Thus, the DNase I hypersensitivity of stem cell DNA is a general phenomenon while the degree of DNase I sensitivity of differentiated cells varies depending on the cell type.

To determine whether the differential DNase I sensitivity of 12-1 and 12-1a cells is a property of chromatin, purified chromatin from 12-1 and 12-1a cells was DNase I digested, and the DNA was isolated and subjected to electrophoresis. Although chromatin of both cell types had been sheared by the isolation procedure (Fig. 1 *F* and *G*, left lanes), the 12-1 chromatin was found to be more sensitive to DNase I digestion (Fig. 1*F*; 2, 4, and 8 min of DNase I digestion) than the chromatin of 12-1a cells (Fig. 1*G*; 2, 4, and 8 min of DNase I digestion).

To determine whether nuclear DNA of 12-1 and 12-1a cells is differentially sensitive to other nucleases, micrococcal nuclease (10 units/mg of DNA) digestion of 12-1 and 12-1a nuclei was carried out. The results of micrococcal nuclease digestion shown in Fig. 1*H* (12-1) and Fig. 1*I* (12-1a) indicate that 12-1 nuclear DNA is also more sensitive to digestion by micrococcal nuclease than is 12-1a nuclear DNA.

DNase I Sensitivity of Plasmid Genomes in Stem and Differentiated Cells. DNase I-digested DNAs of 12-1 and 12-1a cells were cleaved with *Bam*HI, subjected to electrophoresis, and transferred to nitrocellulose paper, and filters were hybridized to ^{32}P-labeled SV40, HSV-1 *tk*, or pBR322 DNA. Re-

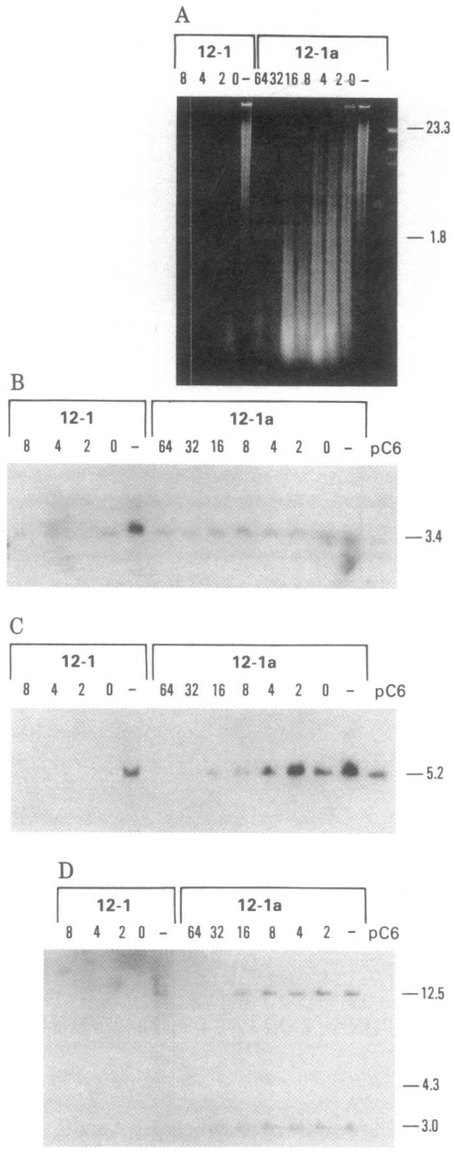

FIG. 2. DNase I sensitivity of plasmid genomes in stem and differentiated cells. 12-1 and 12-1a nuclei were digested with DNase I (10 μg/mg of DNA) [numbers across the top of each figure indicate the time (min) of digestion and those on the right indicate sizes of fragments (kb pairs); minus signs indicate that nuclease was not added], and DNAs were isolated at each time point and digested with *Bam*HI. Agarose gels (*A*) were blotted, and nitrocellulose filters were hybridized with nick-translated ^{32}P-labeled HSV-1 *tk* (*B*), SV40 (*C*), or pBR322 (*D*) DNA.

sults of these experiments are shown in Fig. 2. A representative ethidium bromide-stained agarose gel (Fig. 2*A*) shows the distribution of DNase I/*Bam*HI-digested DNA fragments from the two cell types. Fig. 2*B* shows that the HSV-1 *tk* 3.4-kb fragment is still detectable in 12-1a nuclei after 64 min of DNase I digestion and in 12-1 nuclei after 8 min of DNase I digestion. There is little variation in the HSV-1 *tk* band intensity with increasing time of DNase I digestion of 12-1a nuclei.

The 5.2-kb SV40 genome, which is transcribed in both 12-1 and 12-1a cells (unpublished results), was still detectable in 12-1a cells after 16 min of DNase I digestion (Fig. 2*C*) but was not detectable in 12-1 cells even at the first time point in the experiment (Fig. 2*C*, zero time). When labeled pBR322 DNA was used as the probe (Fig. 2*D*), results were similar to those obtained for the SV40 genome. The pBR322 genome is not transcribed in either 12-1a or 12-1 cells (unpublished results). The pBR322 genome was not detected as a single 4.3-kb band as the pC6 plasmid is integrated into cellular DNA through a site on the pBR322 genome; when the cellular DNA was cleaved with *Bam*HI and analyzed by electrophoresis, Southern transfer, and hybridization with labeled pBR322 DNA, two bands were detected (12.5 and 3.0 kb) that contain pBR322 DNA and host flanking sequences. Thus, in Fig. 2*D*, the disappearance of the 12.5- and 3.0-kb bands with increasing time of DNase I digestion could be due to DNase I-sensitive sites of host flanking sequences, pBR322 sequences, or both.

As the SV40 genome was not intact in 12-1 nuclei after the briefest exposure to DNase I (10 μg/mg of DNA; Fig. 2*C*) we compared the sensitivities of the SV40 genome and the HSV-1 *tk* genome in 12-1 nuclei by using lower DNase I concentrations (Fig. 3). 12-1 nuclei were digested with 1 μg, 0.1 μg, and 0.01 μg of DNase I/mg DNA for 2, 12, and 18 min, and the size distribution of purified DNA fragments was determined by agarose gel electrophoresis (Fig. 3*A*). Each of the DNA samples shown in Fig. 3*A* was then digested with *Bam*HI, subjected to electrophoresis, blotted, and hybridized to labeled HSV-1 *tk* or SV40 DNA. The HSV-1 *tk* genome was detectable at each DNase I concentration at each time point (Fig. 3*C*); the SV40 genome was still intact in 12-1 nuclei after 18 min of digestion with 0.1 (and 0.01) μg of DNase I/mg of DNA but was not de-

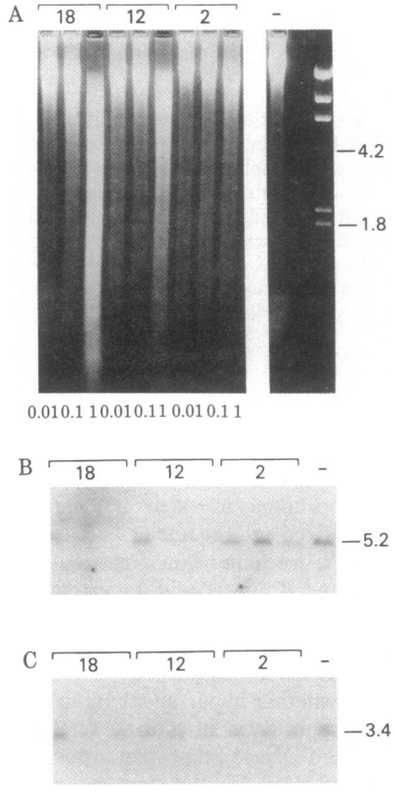

FIG. 3. Sensitivity of SV40 and HSV-1 *tk* genomes in stem cells to low concentrations of DNase I. 12-1 nuclei were digested with DNase I at 1, 0.1, and 0.01 μg/mg of DNA as indicated at the bottoms of the lanes in *A* for 2, 12, and 18 min as indicated at the top of each lane. DNA was isolated for each time point and concentration and subjected to electrophoresis at 10 μg per lane in agarose (0.7%) to determine the size distribution of the fragments (*A*). Parallel DNA samples were digested with *Bam*HI, subjected to electrophoresis, blotted, and hybridized to ^{32}P-labeled SV40 (*B*) or HSV-1 *tk* (*C*) DNA. DNase I concentrations for *B* and *C* are the same as shown in *A*. Numbers on the right indicate fragment sizes (kb pairs); minus sign indicates that nuclease was not added.

tectable at 12 min of digestion with 1 μg of DNase I/mg of DNA when more than 90% of the cellular DNA remained undigested (Table 1).

DNase I Sensitivity of Naked HSV-1 *tk* DNA. As the HSV-1 *tk* genome was insensitive to DNase I digestion in nuclei from both stem and differentiated cells, purified high molecular weight DNA from the two cell types was digested for 0, 2, 8, and 32 min with DNase I (0.1 μg/mg of DNA) and then purified and applied to agarose gels (without restriction enzyme digestion), which were subjected to electrophoresis, blotted, and hybridized to ^{32}P-labeled HSV-1 *tk* DNA. A 3.5-kb pairs band (which did not vary in intensity with cell type or time of digestion) was detected at each time point (except the zero time point) for both cell types, indicating that the naked DNA of the HSV-1 *tk* genome is specifically insensitive to DNase I digestion (data not shown).

DISCUSSION

Chromosomal DNA of teratocarcinoma-derived stem cells is more sensitive to DNase I digestion than is chromosomal DNA of differentiated cells derived from the homologous stem cell. Under the same conditions of digestion, less DNA remains undigested in stem cells than in differentiated cells, and the average molecular weight of resistant DNA fragments is much lower in stem than in differentiated cells, indicating that there are more DNase I-accessible sites in stem cell chromatin. The DNase I hypersensitivity of stem cell chromosomal DNA is not due to endogenous nuclease activity; purified chromatin of stem cells is also hypersensitive to DNase I digestion and DNA isolated from 12-1 nuclei incubated at 37°C for 32 min without DNase I is not significantly degraded. 12-1 chromosomal DNA is also more sensitive to micrococcal nuclease digestion than is 12-1a chromosomal DNA. Hypersensitivity to DNase I digestion is a general property of stem cells; DNase I digestion of nuclei from three stem cells in addition to 12-1 cells gave the same pattern of DNA fragments as 12-1 on electrophoresis. DNase I hyposensitivity of chromosomal DNA is not a general property of differentiated cells; DNA from DNase I-digested F9ACcl9 (a differentiated cell derived from F9) chromosomal DNA has an electrophoretic pattern intermediate between the 12-1 and 12-1a patterns. As F9ACcl9 and 12-1a differentiated cells are both derived from F9 cells, the only genotypic differences between these two cells are that the F9ACcl9 cells have a wild-type murine *TK* gene while the 12-1a cells contain a mutant murine *TK* gene and the pC6 plasmid genome. Phenotypically, the 12-1a cells resemble SV40-transformed fibroblasts and F9ACcl9 cells are parietal endodermal cells, prompting the speculation that the SV40 early region gene products, which are expressed in 12-1a cells, might be responsible, directly or indirectly, for these phenotypic differences, including the differential DNase I sensitivity of chromatin of the two differentiated cell types.

RNA homologous to the early region of the SV40 genome is present in both stem (12-1) and differentiated (12-1a) cells (unpublished results). Thus, the early region of the SV40 genome is transcriptionally active in both cell types and yet the SV40 genome is at least 10-fold more sensitive to DNase I digestion in stem cells (Figs. 2*C* and 3*B*). The pBR322 sequences plus host flanking sequences are also more sensitive to DNase I within stem cell chromatin though pBR322 sequences are not transcribed in stem or differentiated cells. We favor the conclusion that DNase I hypersensitivity of stem cell chromatin is not related to its transcriptional state; that is, the DNase I hypersensitivity of stem cell chromatin has a different configurational basis than the DNase I hypersensitivity of active genes in somatic cells.

The insensitivity of the HSV-1 *tk* gene in stem and differentiated cells is inconsistent with previous studies on DNase I sensitivity of active genes. Our preliminary studies suggest that this insensitivity of the HSV-1 *tk* gene within cellular chromatin is a reflection of DNase I insensitivity of naked HSV-1 *tk* DNA; digestion of 12-1 and 12-1a cellular DNA with DNase I liberates a 3.5-kb pair fragment that hybridizes with HSV-1 *tk* DNA and does not vary in intensity even after 32 min of digestion with DNase I.

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