Journal of Supramolecular Structure 14:85–96 (1980)
Control of Cellular Division and Development: Part B: 115–126

H1 Histone and Nucleosome Repeat Length Alterations Associated With the In Vitro Differentiation of Murine Embryonal Carcinoma Cells to Extra-Embryonic Endoderm

Robert Oshima, Diana Curiel, and Elwood Linney

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 (R.O.); Department of Biology, University of California, San Diego, La Jolla, California 92039 (D.C.); La Jolla Cancer Research Foundation, Torrey Pines Research Center, La Jolla, California 92038 (E.L.)

The histone compositions and average distance between nucleosomes have been determined for F9.22 and PSA1 murine embryonal carcinoma cell lines, for primary extra-embryonic endoderm derived from the in vitro differentiation of PSA1 embryonal carcinoma cells, and for two long-term extra-embryonic endodermal cell lines. A change in the relative proportions of two forms of the H1 histones (H1A and H1B) was found to correlate with the extra-embryonic endodermal differentiated phenotype. The embryonal carcinoma cells had a ratio of H1A/H1B of 1.49 or greater. In contrast, extra-embryonic endoderm from either cell lines or freshly isolated from differentiating embryonal carcinoma cell cultures had a ratio of H1A/H1B of less than 0.9. Partial peptide mapping of gel purified H1A and H1B suggest the two proteins differ in primary structure. The nucleosome repeat length of the embryonal carcinoma cell lines was 196 bp of DNA. Primary extra-embryonic endoderm was found to have a value of 205 bp, but the long-term extra-embryonic endodermal cell lines had an average nucleosome repeat length of 187 bp. Since both freshly isolated primary endoderm and the long-term endodermal cell lines express differentiated functions (basement membrane glycoproteins and plasminogen activator activity), there appears to be no simple correlation between the nucleosome repeat length and the expression of these differentiated functions.

Key words: **embryonal carcinoma, nucleosome repeat length, extra-embryonic endoderm, parietal endoderm, H1 histones, murine teratocarcinoma**

Murine embryonal carcinoma cells are the developmentally pluripotent, malignant stem cells of teratocarcinomas [1–3]. A variety of clonally derived embryonal carcinoma cell lines have been isolated and some of these will differentiate in vitro to a variety of cell

Abbreviations used: DME, Dulbecco's modified Eagle's medium; NP-40, Nonidet P-40 detergent; fbs, fetal bovine serum; EDTA, ethylenediamine tetracetic acid; PBS, phosphate buffered saline; EGTA, ethylene glycol-bis-(β-amino ethyl ether) N,N′-tetracetic acid; PMSF, phenyl methyl sulfonyl fluoride; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin.

Received April 7, 1980; accepted July 16, 1980.

0091-7419/80/1401-0085$03.50

types [4–7]. As a model system of normal development the in vitro differentiation of cultured embryonal carcinoma cells has the advantage of providing relatively large numbers of early differentiated cells for biochemical analysis. Extra-embryonic endoderm is one of the first differentiated cell types to appear during the in vitro differentiation of the PSA1 embryonal carcinoma cell line [5, 8]. These cells can be distinguished from the embryonal carcinoma stem cells by their altered morphology, synthesis of basement membrane glycoproteins [9], secretion of plasminogen activator activity [10], and decreased alkaline phosphatase activity [5, 11]. Thus, we have begun this analysis with extra-embryonic endoderm because of the ease of isolation and identification of these early differentiated cells [9] and the availability of long-term cell lines that express extra-embryonic endodermal functions [12, 13].

Of the five types of histone (H4, H2A, H2B, H3, and H1), the H1 histones are the most heterogeneous in sequence and vary with the type of tissue examined [14, 15]. Because of this variation and the striking changes found in histone subtypes of developing sea urchins [16, 17], we have examined the histone composition of embryonal carcinoma and endodermal cells.* In this report, alterations in the expression of at least two subtypes of the H1 histones are correlated with the endodermal phenotype. In addition, the average nucleosome repeat length has been measured in embryonal carcinoma and endodermal cells from several sources. There does not appear to be a simple correlation between the nucleosome repeat length and the expression of endodermal differentiated functions or the H1 histone composition.

MATERIALS AND METHODS

Dulbecco's modified Eagle's medium (DME, high glucose formulation, 4.5 g glucose/liter) and trypsin were purchased from GIBCO. Selected batches of fetal bovine serum were purchased from Irvine Scientific. ^{3}H-lysine (60–80 Ci/mmole) and ^{3}H-leucine (45 Ci/mmole) were obtained from New England Nuclear. Micrococcal nuclease was purchased from Sigma and Staphylococcus aureus protease V8 from Miles Laboratories. Nonidet P-40 and Triton X-100 detergents were purchased from Sigma.

Cell Culture

The F9.22 [18] embryonal carcinoma, PYS2 [12] and PF HR9 [13] extra-embryonic endodermal cell lines were cultivated in DME supplemented with 1 mM pyruvate, 2mM glutamine, and 10% fetal bovine serum (fbs) in a humidified atmosphere of approximately 90% air, 10% CO_2. The PSA1 embryonal carcinoma cell line was cultivated on mitomycin C-treated mouse fibroblast feeder layers [5] in the same medium supplemented with 10^{-4}M 2-mercaptoethanol [18]. Embryoid body formation and the isolation of primary extra-embryonic endoderm was performed as previously described [9]. Purity of primary endoderm was determined by reaction with teratocarcinoma basement membrane antibodies [9].

When necessary, cells were labeled with either ^{3}H-leucine or ^{3}H-lysine at 25 μCi/ml for 12–18 h in DME containing 1/10 the normal leucine or lysine concentration and supplemented with 10% fetal bovine serum, penicillin, and streptomycin.

*Through the remainder of this report "extra-embryonic endoderm" may be referred to as simply "endoderm," however, this should not be confused with definitive embryonic endoderm, which appears much later in development.

Isolation of Nuclei

Two procedures were used. In the first, cells were harvested by brief treatment of PBS-washed cultures with isotonic 0.05% trypsin, 0.025% EDTA. Media containing 10% fbs was used to stop the action of the trypsin and EDTA after 3–5 min at 37°C. After washing in DME 10% fbs and in PBS at 4°C, the cells were suspended in 0.5% NP-40, 0.25 M sucrose, 0.1 mM EGTA, and 0.5 mM phenylmethyl sulfonyl fluoride (PMSF) in RSB buffer (10 mM Tris-HCl, pH 7.2, 10 mM NaCl, 3 mM $MgCl_2$) at less than 3×10^7 cell/ml. The cells were disrupted by Dounce homogenization monitored by phase microscopy. The nuclear fraction was recovered by centrifugation through a cushion of 30% sucrose, 0.1 mM EGTA in RSB at approximately 750g for 10 min at 4°C. The homogenization and centrifugation steps were repeated. The nuclei were then resuspended in digestion buffer (0.25 M sucrose, 0.1 mM $CaCl_2$ in RSB) and centrifuged through a cushion of 30% sucrose, containing the same ions. The nuclei were resuspended in digestion buffer at a concentration that yielded an absorbance at 260 nm of 20 in 1.0 M NaOH.

An alternate procedure for the isolation of nuclei without the use of trypsin involved rinsing monolayers of cells with cold PSB followed by lysis in situ by the addition of 0.5% NP-40, 0.25 M sucrose, 0.1 mM $CaCl_2$, 0.5 mM PMSF, 5 mM N-ethylmaleimide (NEM) in RSB. The nuclei and cytoskeletons were removed by scraping and recovered by centrifugation at approximately 750g for 5 min. The pellet was suspended in the lysis buffer used for whole cells and processed as described for the first procedure. The histones of nuclei isolated by this method appeared identical to those isolated by the first method, as judged by electrophoretic analysis.

Histone Isolation and Analysis

Nuclei were digested with micrococcal nuclease (25 μg/ml) for 10 min at 37°C. The reaction was stopped by the addition of EDTA to 10 mM. Cold NaCl was added to a final concentration of 1M, and the nuclei were incubated on ice for 10–15 min with occasional mixing. Nonhistone proteins were precipitated by the addition of HCl to 0.25 M. After centrifugation at 10,000g for 10 min at 2°C, the supernatant was removed and dialyzed against either 0.1% SDS at room temperature or 0.1 mM PMSF, 10^{-4} M 2-mercaptoethanol in the cold. The samples were lyophilized and redissolved in the appropriate electrophoresis sample buffer. The histones were analyzed on 1 mm-thick, 25 cm-long polyacrylamide gels according to the procedures of Thomas and Kornberg [19] for gels containing SDS or Zweidler [20] for gels containing Triton X-100, urea, and acetic acid. Gels were stained with 0.1% Coomassie blue R-250 in 50% methanol and 10% acetic acid and destained in 10% methanol and 10% acetic acid. Gels containing radioactive samples were processed for fluorography [21] and exposed to preflashed [22] Kodak XR5 X-ray film.

Alkaline phosphatase treatment of F9.22 histones was performed in 25 mM Tris-HCl, pH 8, at 37°C for up to 3 h with 500 μg/ml of enzyme and approximately 34 μg/ml of ^{3}H-lysine labeled histone.

H1A and H1B were purified by preparative electrophorsis in SDS on 3 mm-thick, 25 cm-long gels. The gel was stained briefly with Coomassie blue in 50% methanol and 10% acetic acid. The bands were excised with a razor blade and the proteins eluted electrophoretically into dialysis tubing. The eluted protein was dialyzed against 0.1% SDS, lyophilized, redissolved in 1/10 volume of H_2O, and precipitated with either TCA (20% final concentration) or 9 volumes of acetone. The pellets were redissolved in 0.5% SDS, 62.5 mM Tris-HCl, pH 6.8. Protein concentration was determined by the method of Lowry et al [23]

with BSA in 0.1% SDS as the standard. Proteolytic cleavage in SDS was performed with Staphylococcus aureus protease V8 as described by Cleveland et al [24]. Peptides were analyzed in 15% acrylamide, 0.4% bis-acrylamide gels in the buffers described by Thomas [19], with 0.1 mM EDTA added to both stacking and resolving gels.

Nucleosome Repeat Analysis

Nuclei were digested with micrococcal nuclease (25 μg/ml) for either 30 or 60 sec at 37°C. The reaction was stopped by the addition of EDTA to 10 mM and SDS to a final concentration of 1%. DNA samples were prepared by proteinase-K treatment, phenol extraction, and RNase treatment as described by Gross-Bellard et al [25]. Samples (50 μl) containing approximately 10 μg of DNA were subjected to electrophoresis on 3 mm-thick, 23 cm-long vertical slab gels of 0.5% agarose, 2.5% acrylamide (19:1 acrylamide:bis-acrylamide) in Loening's buffer [26]. Electrophoresis was performed at 60 V for 16 h. Gels were stained in tray buffer with 0.5 μg/ml ethidium bromide for 40 min, trans-illuminated with ultraviolet light, and photographed with Polaroid type 55 film. Negatives were scanned with a Joyce-Loebl microdensitometer. The nucleosome repeat length was estimated by linear regression analysis of plots of the mean size of the DNA bands in base pairs vs the band number [27].

RESULTS

Histones isolated from embryonal carcinoma and endodermal nuclei were analyzed by electrophoresis in the presence of SDS. Figure 1 shows the histone profile of F9.22 embryonal carcinoma cells and the PF HR9 endodermal cell line. It is apparent that the two forms of the H1 histones resolvable by this gel system vary in their relative proportion depending on the cell line. This variation of H1A and H1B is correlated with the differentiated phenotype of the cell as shown in Table I. Three embryonal carcinoma cell lines had a ratio of H1A/H1B of 1.49 or greater. In contrast, endodermal cells from either long-term cell lines or isolated from differentiated PSA1 embryonal carcinoma cultures had less H1B than H1A. Approximately 90% or more of each endodermal cell preparation in Table I reacted unambiguously with antiserum directed against basement membrane glycoproteins [9]. The FOT 5 and F9.22 embryonal carcinoma cell lines were contaminated with less than 1% of cells reactive with the basement membrane antiserum. The PSA1 embryonal

TABLE I. H1 Histone Composition of Embryonal Carcinoma and Endodermal Cells

Cell line	Phenotype	Method of detection	H1A/H1B[a]
FOT 5	Embryonal carcinoma	Coomassie blue	1.98
F9.22	Embryonal carcinoma	^{3}H-Leucine, fluorography	1.70
PSA1	Embryonal carcinoma	^{3}H-Lysine, fluorography	1.49
PSA1-ENDO	Primary endoderm	^{3}H-Lysine, fluorography	0.85
PSA1-ENDO	Primary endoderm	^{3}H-Leucine, fluorography	0.80
PYS 2	Endodermal line	^{3}H-Leucine, fluorography	0.87
PF HR9	Endodermal line	^{3}H-Lysine, fluorography	0.30
PF HR9	Endodermal line	Coomassie blue	0.31

[a]Values represent the ratio of the areas of the 2 bands determined by scanning with a densitometer either the stained gel or preflashed X-ray film exposed to gels processed for fluorography as described by Bonner and Laskey [21]. The area was determined by cutting out the peaks and weighing the paper.

carcinoma cell line, which spontaneously differentiates in vitro, contained less than 10% reactive cells [9].

The relative migration positions of F9.22 H1A and H1B were unaffected by prior treatment with a 12-fold excess of E coli alkaline phosphatase. This suggests that phosphorylation is not responsible for the two forms of H1. Several observations confirm that the H1 differences between embryonal carcinoma cells and extra-embryonic endodermal cells are not artifacts of partial proteolysis during the histone isolation procedure:

1. PF HR9 cells labeled with ^{3}H-lysine were mixed with a 10-fold excess of non-radioactive F9.22 cells. Nuclei were isolated and histones were extracted and analyzed by electrophoresis. After staining with Coomassie blue, the relative intensities of the H1 bands were as expected for F9.22 cells. However, after fluorography the radioactive profile of the H1 bands resulted in the value for ^{3}H-lysine-labeled PF HR9 listed in Table I.

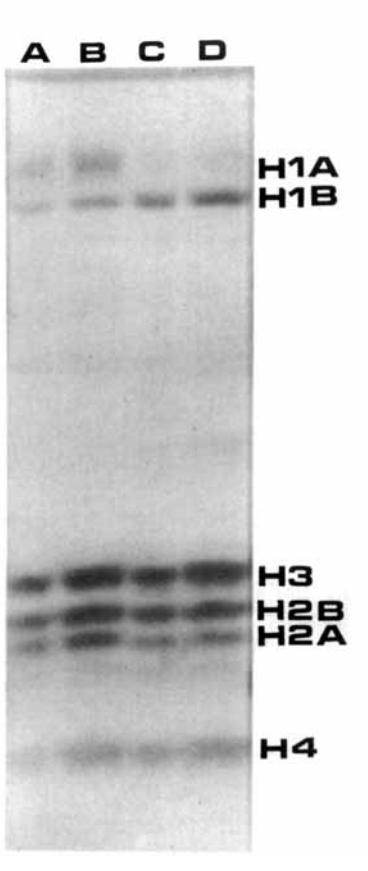

Fig. 1. Comparison of F9.22 embryonal carcinoma and PF HR9 endodermal histones. Nuclei were isolated from the two cell types, digested for 10 min with micrococcal nuclease then extracted with 1M NaCl. Nonhistones were precipitated with 0.25 M HCl. The supernatant was dialyzed against 0.1 mM PMSF and 10^{-4}M mercaptoethanol in the cold, lyophilized, and analyzed on 24 cm-long acrylamide gels (15% acrylamide, 0.09% bis-acrylamide) in 0.1% SDS as described by Thomas [19]. A: F9:22, 5 μg; B: F9.22, 10 μg; C: PF HR9, 5.8 μg; D: PF HR9, 11.6 μg.

2. The relative intensities of the H1 subtypes identified in samples of whole nuclei were the same as those found for extracted histones. In addition, electrophoretic analysis of the residue left after extraction of the histones revealed no histone. Thus, the differences in H1 subtypes are not due to incomplete extraction.

3. Preparation of histones without the use of proteolytic enzymes (see Materials and Methods) resulted in H1 histone profiles identical with those shown in Figure 1.

The two forms of H1 found in embryonal carcinoma cells were isolated by preparative electrophoresis and subjected to partial proteolysis by Staphylococcus protease V8 according to the procedures of Cleveland et al [24]. Figure 2 shows the migration pattern of the isolated H1A and H1B and the peptides generated by the action of the protease. By comparison of lanes H and I, with J and K, it is evident that H1A is more resistant to the action of the protease than H1B. At the highest protease concentration used (lanes F and

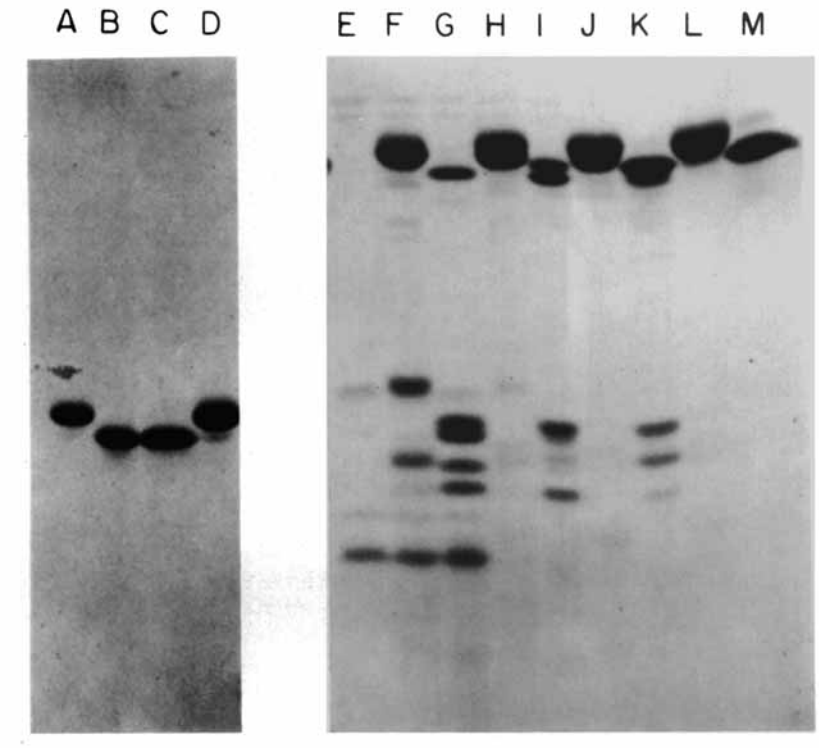

Fig. 2. Partial peptide maps of F9.22 H1A and H1B. Histones H1A and H1B were purified by preparative SDS gel electrophoresis of the acid-soluble extracts of F9.22 embryonal carcinoma nuclei. The eluted proteins were then rerun on a 15% acrylamide gel containing 0.09% bis-acrylamide to verify their purity. A: H1A; B: H1B; C: H1B; D: H1A. The eluted proteins were digested for 4 h at 37°C in 0.5% SDS, 62.5 mM Tris-HCl, pH 6.8, 10% glycerol at 0.5 mg/ml with the indicated concentration of Staphylococcus aureus V8 protease according to the procedure of Cleveland et al [24]. The digests were analyzed in 15% acrylamide and 0.4% bis-acrylamide slab gels in the buffers described by Thomas [19]. E: 272 μg/ml V8 protease with no histones added; F: H1A, 272 μg/ml V8; G: H1B, 272 μg/ml V8; H: H1A, 54 μg/ml V8; I: H1B, 54 μg/ml V8; J: H1A, 11 μg/ml V8; K: H1B, 11 μg/ml V8; L: H1A; M: H1B.

G), several peptide differences are obvious between H1A and H1B. Although it is still possible that more than one polypeptide species is represented by the H1A or H1B bands, the partial peptide maps suggests that the major polypeptide(s) of H1A and H1B differ in amino acid sequence. An identical analysis of H1A and H1B isolated from the PF HR9 endodermal cell lane yielded very similar results (data not shown).

The core histones (H4, H2A, H2B, H3) can be resolved further into subtypes by electrophoresis in acrylamide gels containing urea, acetic acid, and Triton X-100 [20]. To determine whether the subtypes of the core histones were altered when embryonal carcinoma cells differentiated to endoderm, extracted histones from the two cell types were analyzed in 25 cm-long, 12% acrylamide gels containing 7.5 M urea, 5% acetic acid, and 6 mM Triton X-100 (Fig. 3). These conditions resolve H2A and H2B each into 2 distinct

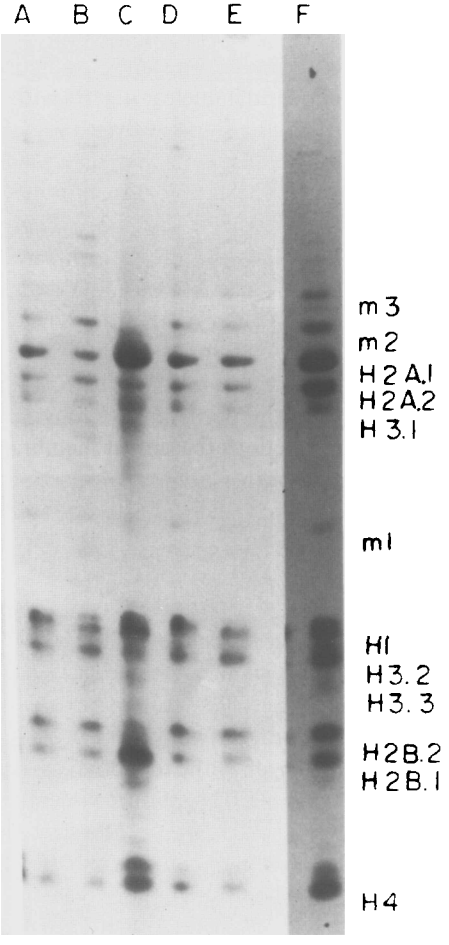

Fig. 3. Electrophoretic analysis of embryonal carcinoma and endodermal histones in a polyacrylamide (12% acrylamide, 0.08% bis-acrylamide) slab gel containing 6 mM Triton X-100, 7.5 M urea, and 5% acetic acid [20]. Approximately 12 μg of histones were loaded in each lane. A: FOT 5 embryonal carcinoma; B: PF HR9 endoderm; C: commercial calf-thymus histones; D: F9.22 embryonal carcinoma; E: PYS 2 endoderm; F: 21-day exposure of ^{3}H-leucine labeled PSA1 endodermal histones, isolated in the same gel as the other samples but processed for fluorography.

subtypes. However, no differences in these subtypes (H2A.1, H2A.2, H2B.1, H2B.2) were observed between embryonal carcinoma and endodermal cells. In addition, three subtypes of H3 and acetylated forms of H4 were detected in these gels, but again no differences between embryonal carcinoma and endodermal cells were obvious.

When isolated nuclei are subjected to limited digestion by micrococcal nuclease; a series of DNA fragments are generated that correspond to the DNA associated with the repeating nucleosome unit. The average length of DNA between nucleosomes is determined by comparing the size of the DNA fragments to the number of nucleosomes the fragments represent [28]. The average distance between nucleosomes (or nucleosome repeat length) of embryonal carcinoma and endodermal nuclei was determined by digesting isolated nuclei briefly with micrococcal nuclease, isolating the DNA, and determining the size of the DNA fragments by electrophoresis in agarose-acrylamide gels with ΦX174 DNA restriction fragments [40] as size standards. Figure 4 shows representative microdensitometer tracings of an agarose-acrylamide gel after electrophoresis of DNA from embryonal carcinoma and endodermal cell line nuclei, briefly digested with micrococcal nuclease. Figure 5 shows a comparison of the size in base pairs of the multiple DNA bands isolated from embryonal carcinoma and endodermal nuclear digests with the band number. The average repeat length is estimated from the slope of these plots. Figure 5A shows that a small but consistent difference can be detected between the nucleosome repeat lengths of embryonal carcinoma and endodermal nuclei. In contrast, no discernable difference in nucleosome repeat length can be detected if the two embryonal carcinoma cell lines are compared (Fig. 4B), or if the two endodermal cell lines are compared (Fig. 4C). The results of several experiments are summarized in Table II. The endodermal cell lines tested had consistently shorter repeat lengths than the embryonal carcinoma cells. However, endoderm freshly isolated from differentiated PSA1 embryonal carcinoma cell cultures appeared to have a slightly longer repeat length than the embryonal carcinoma cells from which they were derived. Since both the primary endoderm and the long-term endodermal cell lines express endodermal differentiated functions (basement membrane glycoprotein antigens and plasminogen activator secretion), there does not appear to be a strict correlation between the average nucleosome repeat length and the expression of these differentiated functions.

DISCUSSION

When the PSA1 embryonal carcinoma cell line is permitted to differentiate in vitro, structures called embryoid bodies form which resemble early mouse embryos [8]. The first differentiated cell type to appear in such cultures is extra-embryonic endoderm [5, 8]. In the mouse, extra-embryonic endoderm is composed of two kinds of cells. Parietal endoderm synthesizes and secretes plasminogen activator activity [29] and the basement membrane known as Reichart's membrane [30, 31]. Mature visceral endoderm, the other cellular component of extra-embryonic endoderm, synthesizes alpha-fetoprotein [32]. Antiserum directed against the basement membrane-like material secreted by embryoid bodies reacts strongly with authentic parietal endoderm dissected from embryos and to a lesser extent visceral endoderm [9, 33]. The primary endodermal cells isolated from PSA1 embryoid bodies used in this study react strongly with this antiserum and secrete plasminogen activator activity [9, 10]. However, since PSA1 embryoid bodies eventually produce alpha-fetoprotein in vitro [34], it is possible that the primary differentiated cells isolated from embryoid bodies may contain some immature visceral endoderm. The long-term endodermal cell lines used in this study do not synthesize significant quantities of alpha-

fetoprotein but do secrete plasminogen activator and basement membrane glycoproteins. Although these cell lines are convenient sources of pure differentiated cell populations (probably most representative of parietal endoderm), it must be assumed that they have gone through some evolutionary transition analogous to that documented for the establishment of fibroblastic cell lines [35]. If the difference in average nucleosome repeat length between the primary-isolated endoderm (205 bp) and the endodermal cell lines (187 bp) is significant, it may be related to the transition of a primary differentiated cell population to an established cell line. The small difference in nucleosome repeat length between the PSA1 embryonal carcinoma cell line (196 bp) and the primary endoderm

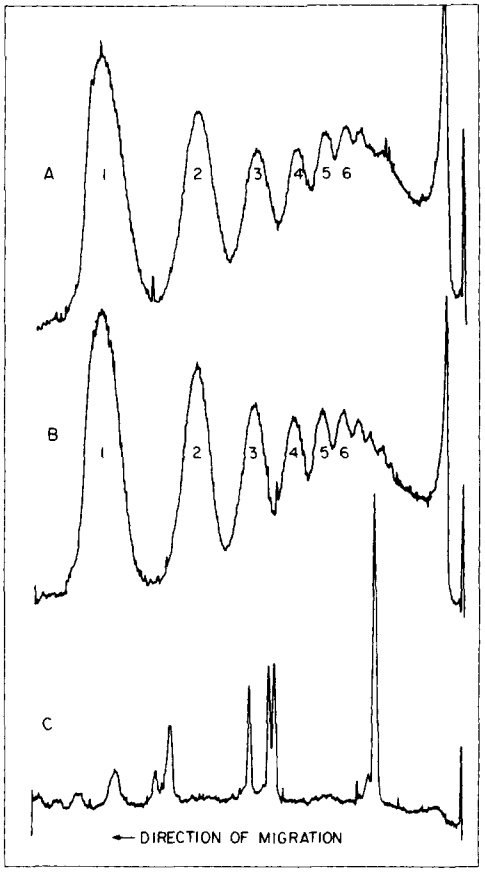

Fig. 4. Size determination of DNA fragments resulting from the digestion of embryonal carcinoma and endodermal nuclei with micrococcal nuclease. Isolated nuclei resuspended to A_{260} = 20, were digested with 25 μg/ml micrococcal nuclease in 0.25 M sucrose, 10 mM Tris-HCl, pH 7.2, 10 mM NaCl, 3 mM $MgCl_2$, 0.1 mM $CaCl_2$, at 37°C. The reaction was stopped with the addition of EDTA to 10 mM. DNA were prepared by treatment with proteinase K; phenol extraction and RNase treatment was performed as described by Gross-Bellard et al [25]. Approximately 10 μg of DNA of each sample were electrophoresed on 3 mm-thick, 23 cm-long vertical slab gels of 0.5% agarose 2.5% acrylamide in Loening's buffer for 16 h at 60 V. Gels were same as above UV light transillumination. Polaroid type 55 film negatives were scanned on a Joyce Loebl microdensitometer. A; F9.22, digested for 1 min; B: PF HR9 nuclei, digested for 1 min; C; ΦX174 Hae III fragments.

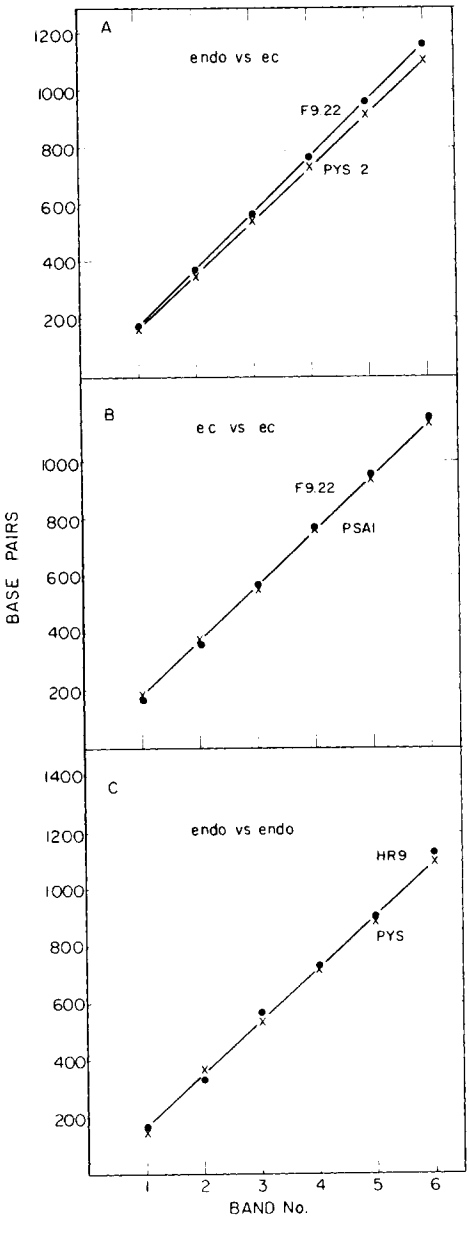

Fig. 5. Comparison of the nucleosome repeat length of embryonal carcinoma and endodermal nuclei. The sizes of the DNA fragments generated by digestion of nuclei with micrococcal nuclease for 1 min were determined by electrophoresis. The size of the midpoint of each band is compared with the band number starting with the smallest molecular weight band. The slope of the line indicated the average nucleosome repeat distance. A: •, F9.22 embryonal carcinoma cells; X, PYS 2 endodermal cells. B: •, F9.22 embryonal carcinoma cells; X, PSA1 embryonal carcinoma cells. C: • PF HR9 endodermal cells, X, PYS 2 endodermal cells.

TABLE II. Average Nucleosome Repeat Length of Murine Embryonal Carcinoma and Endodermal Cells*

Cell line	Phenotype	Experiment 1	2	3	Average
F9.22	Embryonal carcinoma	203	194, 191	–	196 ± 6
PSA1	Embryonal carcinoma	–	191, 194	200	195 ± 5
PSA1 ENDO	Primary endoderm	–	–	205, 204	205
PYS2	Endodermal cell line	183	188, 184	190	185 ± 3
PF HR9	Endodermal cell line	–	188, 183	189	187 ± 3

*Each value represents independently prepared samples. All samples of each experiment were run on the same gel. The size in base pairs of DNA of each band was determined by comparing the migration distance to that of ΦX174 restructuib fragments (Hae III and Hha 1) [40]. The repeat lengths were determined by linear regression analysis of the band number vs the size of the band. The standard deviation of any single value was ± 1 bp.

(205 bp) isolated from differentiating PSA1 cultures is consistent with previous reports of longer repeat lengths generally associated with the less actively dividing tissue. Non-dividing cells such as sperm and nucleated erythrocytes have longer repeat lengths, as do myotubes when compared with myoblasts [36]. However, a simple correlation between growth rate and repeat lengths of cultured cells is not found [36]. Since both primary isolated endoderm and endodermal cell lines contain basement membrane glycoproteins and secrete plasminogen activator activity, the expression of these differentiated functions does not appear to be strictly correlated with the average nucleosome repeat length.

The differential expression of the H1 multigene family appears to be correlated with the differentiated cell phenotype. (Table I) The results of the partial peptide mapping (Figure 2) suggests that the two forms of H1 resolvable by electrophoresis in SDS represents at least two different polypeptide sequences. Preliminary results of the amino acid compositions of H1A and H1B support this view. (The lysine/arginine ratio of H1A is greater than that of H1B) The H1 histones are known to be the most diverse of the mammalian histones [15] and tissue-specific variation in the amounts of chromatographically resolvable H1 subtypes have been reported [14]. However, the best documented cases of tissue-specific variation have involved tissues that contain several types of cells [37]. Since three H1 subtypes have been reported for fibroblast mouse cell lines [38], it appears that additional changes in H1 histone expression might be expected to appear during subsequent transitions in the differentiation of embryonal carcinoma cultures to mesodermal derivatives. The H1 histones appear to bind the linker DNA between nucleosomes [15, 28] and may play a role in higher orders of chromatin condensation. It is tempting to speculate that H1 may play a role in the condensation of specific regions of chromatin that are not expressed in a particular differentiated cell type [39], but no evidence yet exists for the specific association of H1 subtypes with particular regions of chromatin. The strategy of examining embryonal carcinoma-derived differentiated cell populations as a model of normal embryonic development may provide the necessary material for the development of immunological and molecular probes specific for particular differentiated cell types such as parietal endoderm. The development of such specific probes could then be applied to the analysis of developing mouse embryos to confirm observations made in the teratocarcinoma system.

ACKNOWLEDGMENTS

The authors thank Maria Felshtinsky for technical assistance and Deborah Hagman for typing the manuscript. This work was supported by grants CA 27580 (RGO) and CA 24206 (EL) from the National Cancer Institute, NIH and grant BC 314 (RGO) from the American Cancer Society. E.L. was assisted by a Leukemia Society of America scholarship.

REFERENCES

1. Kleinsmith LJ, Pierce GB: Cancer Res 24:1544, 1964.
2. Hogan BLM: In Paul J (ed): "International Review of Biochemistry, Biochemistry of Cell Differentiation II." Baltimore: University Park Press, 1977, vol 15.
3. Martin GR: Cell 5:229, 1975.
4. Jakob H, Boon T, Gaillard J, Nicholas JF, Jacob F: Ann Microbiol (Paris) 124B:269, 1973.
5. Martin GR, Evans MJ: Proc Nat Acad Sci USA 72:1441, 1975.
6. McBurney MW: J Cell Physiol 89:441, 1976.
7. Martin GR, Evans MJ: Cell 6:467, 1975.
8. Martin GR, Wiley LM, Damjanov I: Dev Biol 61:230, 1977.
9. Oshima RG, Linney E: Exp Cell Res 126:485, 1980.
10. Linney E, Levinson BB: Cell 10:297, 1975.
11. Bernstine EG, Hooper ML, Grandchamp S, Ephrussi B: Proc Nat Acad Sci USA 70:3899, 1973.
12. Lehman JM, Speers WC, Swartzendruber DE, Pierce GB: J Cell Physiol 84:13, 1974.
13. Chung AE, Estes LE, Shinozuka H, Baginski J, Lorez C, Chung CA: Cancer Res 37:2072, 1977.
14. Bustin M, Cole RD: J Biol Chem 243:4500, 1968.
15. Elgin SCR, Weintraub H: Ann Rev Biochem 44:725, 1975.
16. Ruderman JV, Gross PR: Dev Biol 36:286, 1974.
17. Cohen LH, Newrock KM, Zweidler A: Science 190:994, 1975.
18. Oshima RG: Differentiation 11:149, 1978.
19. Thomas JO, Kornberg R: Proc Nat Acad Sci USA 72:2626, 1975.
20. Zweidler A: Meth Cell Biol 17:223, 1977.
21. Bonner WM, Laskey RA: Eur J Biochem 46:83, 1974.
22. Laskey RA, Mills AD: Eur J Biochem 56:335, 1975.
23. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ: J Biol Chem 193:265, 1951.
24. Cleveland D, Fisher S. Kirshner M, Laemmli U: J Biol Chem 252:1102, 1977.
25. Gross-Bellard M, Oudet P, Chambon P: Eur J Biochem 36:32, 1973.
26. Loening UE: Biochem J 102:251, 1967.
27. Thomas JO, Thompson RJ: Cell 10:633, 1977.
28. Noll M, Kornberg RD: J Mol Biol 109:393, 1977.
29. Strickland S, Reich E, Sherman MI: Cell 9:231, 1976.
30. Pierce GB, Midgley AR, Sri Ram J, Feldman JD: Amer J Path 41:549, 1962.
31. Pierce GB: Dev Biol 13:231, 1966.
32. Dziadek M, Adamson E: J Embroyol Exp Morph 43:289, 1978.
33. Jetten AM, Jetten MER, Sherman MI: Dev Biol 70:89, 1979.
34. Adamson EI, Evans MJ, Magrane GG: Eur J Biochem 79:607, 1977.
35. Todaro GJ, Green H: J Cell Biol 17:299, 1963.
36. Compton JL, Bellard M, Chambon P: Proc. Nat Acad Sci USA 73:4382, 1976.
37. Rall SC, Cole RD: J. Biol Chem 246:7175, 1971.
38. Ajior K, Zweidler A, Borun T, Croce C: Proc. Nat Acad Sci USA 75:5599, 1978.
39. Simpson RT, Reeck GR: Biochem 12:3853, 1973.
40. Jeppeson PGN, Sanders L, Slocombe PM: Nucleic Acids Res 3:1323, 1976.