Histone Gene Expression and Chromatin Structure in Mammalian Cell Hybrids

NANCY HSIUNG and RAJU KUCHERLAPATI
Department of Biochemical Sciences, Princeton University, Princeton, New Jersey 08540

ABSTRACT

DNA isolated from mammalian cell nuclei reveals discrete size patterns when partially digested with micrococcal nuclease. The DNA repeat lengths from different tissues within a species or from different species may vary. These differences have been attributed to the presence of different species of histone H1. To examine the nature of regulation of DNA repeat lengths and their possible relationship to histone H1, we have selected several mouse and human cell lines that differ in their DNA repeat lengths and examined them and their cell hybrids. 24 mouse × human and five mouse × mouse hybrid cell lines were analyzed. All the interspecific hybrids exhibited the repeat pattern characteristic of the murine parent. The mouse intraspecific hybrids had a repeat pattern of only one of the parents. We conclude that the partial human chromosome complements retained in the hybrids assume the repeat lengths exhibited by the mouse cells.

Because H1 histones have been implicated in the determination of DNA repeat lengths, we also investigated the regulation of H1 histone expression in these cell hybrids. Purified H1 histones were radioactively labeled in vitro, and individual subfractions were subjected to proteolysis followed by gel electrophoresis. The resulting partial peptide maps of H1 histone subfractions A and B were distinguishable from one another and from different cell lines. In the mouse × human hybrids analyzed, only the mouse H1 histones were detected. These observations were extended to H2b by analysis of the hybrid cell histone by Triton-acid-urea gels. Neither the DNA repeat length nor histone expression is affected by the presence of any specific human chromosome. The fact that human genes are expressed in these hybrids suggests that the H1 histones of one species is able to interact with the chromatin of another species in a biologically functional conformation.

Analysis of the intraspecific PG19 × B82 (mouse × mouse) hybrids reveals the presence of H1 histone subfractions of the B82 mouse cells. Because these hybrids exhibit the nucleosome repeat length only of the PG19 cells, it appears that if histone H1 plays a role in determining the repeat length it does so in consort with other nonhistone chromosomal proteins.

The unit of chromatin structure is the nucleosome, a DNA-histone complex repeated approximately every 200 base pairs, as first seen by electron microscopy (33, 42) and later by digestion of nuclei with micrococcal nuclease (4, 17, 31, 36). Although this repeating structure is consistently found, the nucleosome repeat size or DNA repeat length is different in different cell types, tissues, and organisms (10, 27, 29, 30, 32, 37, 40). Though the differences in repeat length seen by digestion with micrococcal nuclease is well documented, the functional significance of the variability is not understood. Therefore, the study of the possible genetic basis for DNA repeat lengths is of interest.

The conformation of chromatin structure appears to be different in various functional states of cells (5, 14, 16, 41). These conformational changes in chromatin structure strongly depend on the histone-DNA interaction. Histones H2a, H2b, H3, and H4 are highly conserved and form the nucleosome core particle (see references 11 and 23 for review). The least conserved histone, H1, appears to associate with the outside of the core nucleosome. The existence of H1 variants (see reference 19 for review) and the expression of the distinct H1 histone subfractions during specific developmental stages (3, 9, 18) implicate this protein in the regulation of gene expression. A number of studies (29, 30, 32, 37) have suggested the
involvement of H1 histone in determining nucleosome repeat lengths. The differences in repeat length were ascribed to the spacer or linker region between the nucleosomal core particles. In fungi (29, 32), where short DNA repeat sizes were observed, the H1 histones in both Neurospora crassa (15) and Aspergillus nidulans have fewer basic residues than those found in higher eukaryotes (13), suggesting that the shorter repeat lengths in these organisms are probably caused by the existence of fewer basic groups to interact with the DNA phosphate groups in the spacer region. Similarly, during chick erythropoiesis, the increase in nucleosome repeat length from 190 to 212 base pairs (40) occurs with the concomitant increase in the more basic H5 histone expression.

To study the possible genetic basis for the expression of different DNA repeat lengths in different systems, the expression of H1 histones, and the interrelationship between H1 histone and nucleosome repeats, we have employed somatic cell genetic techniques and conducted the following experiments. We screened different rodent and human cell lines to select those cells that contain distinguishable DNA repeat lengths and distinct H1 histone subfractions. A number of mouse × human and mouse × mouse hybrids were analyzed to study the regulation of the expression of various DNA repeat lengths and H1 histones. We report here that in each set of hybrids the repeat length of only one of the parental lines is observed. In the mouse × human hybrids, the expression of H1 is also regulated; only the H1 histone of the mouse parent is expressed. The observation that these hybrids express several human genes suggests that the human chromatin can associate with the mouse H1 histones and retain a functionally active conformation. Finally, results from the mouse × mouse hybrids imply that H1 alone is not directly responsible for determining the repeat size in chromatin structure.

Materials and Methods

Cell Lines

Parental Cells: GM1429 cells, human diploid fibroblasts; B22, a permanent mouse L cell derivative deficient in thymidine kinase; and A9, a permanent mouse L cell derivative deficient in hypoxanthine phosphoribosyl transferase, were all obtained from the Institute for Medical Research, Camden, N. J. HT1080, a human fibrosarcoma line, and Hela S3, a human epithelial carcinoma line, were obtained from the American Type Culture Collection, Rockville, Md. PG19, a mouse melanoma derivative deficient in hypoxanthine phosphoribosyl transferase, was provided by R. Kennett, University of Pennsylvania. All cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Grand Island Biological Co. [GIBCO], Grand Island, N. Y.) and supplemented with 10% fetal bovine serum (Flow Laboratories, Inc., Rockville, Md.), penicillin and streptomycin (GIBCO), and glutamine (GIBCO).

Hybrid Cells: PEP hybrids are the result of fusion between PG19 and BP (diploid human fibroblasts) cells. MGM hybrids were obtained from fusion between PG19 and GM1429 cells. Fusion between B22 and HT1080 cells resulted in BCH hybrids. The production method and characteristics of the PEP, MGM, and BCH hybrids are described by Kucherlapati et al. (24). The PGP hybrids were obtained by polyethylene glycol-mediated fusion of PG19 and B22 cells (Tepper and Kucherlapati, unpublished data). A9 × DUV (diploid human fibroblasts) resulted in DUA hybrids (35).

Preparation of Nuclei and Nuclear Digestions: Cells were pelleted, washed, and resuspended in 0.3% Nonidet P-40 (NP-40) in RSB (10 mM Tris-Cl, pH 7.4, 10 mM NaCl, 5 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride). After 5 min, the cells were lysed in a Dounce homogenizer (8 pestle). Nuclei were isolated by centrifugation, washed, and finally resuspended at a concentration of 1.5 mg/ml in 0.5% NP-40 in reticulocyte suspension buffer (RSB) containing 0.5-1 mM CaCl₂, Micrococcal nuclease (Worthington Biochemical Corp., Freehold, N. J.) (150 U/ml) was added, and digestion was carried out at 37°C for increasing periods of time. The digestion was terminated by the addition of 0.1 M EDTA, pH 7.1, to a final concentration of 10 mM at 4°C. The digested nuclei were pelleted by centrifugation, resuspended in RSB buffer containing 0.5% NP-40, and treated with Ribonuclease A (Sigma Chemical Co., St. Louis, Mo.) (40 ng/ml) at 37°C for 30 min, followed by incubation at 37°C for at least 2 h in the presence of 200 µg/ml of Pronase (Calbiochem-Behring Corp., American Hoecht Corp., San Diego, Calif.) and 0.5% SDS. The DNA samples were then added to an equal volume of sample buffer (50% glycerol, 0.005% bromophenol blue, 40 mM Tris-acetate, pH 7.8, 20 mM sodium acetate, and 2 mM EDTA). No difference in results was observed when the DNA was isolated by phenol/chloroform extraction, followed by ethanol precipitation.

Preparation of Chromatin and Isolation of H1 or Total Histones: Nuclei were resuspended at a concentration of 1.5 mg/ml in 0.5% NP-40 in RSB containing 1 mM CaCl₂ and digested for 5 min at 37°C with 100 U/ml of micrococcal nuclease. The nuclei were pelleted by centrifugation and then lysed in 5 mM EDTA. Nuclear debris was removed by low-speed centrifugation. Extraction of H1 histones was carried out by the addition of 15% SDS polyethylene glycol to a final concentration of 5%. After at least 30 min at 4°C, the proteins were fractionated by centrifugation. H1 histones were retained in the soluble fraction and concentrated by precipitation in 22% trichloroacetic acid. Total histones were isolated from nuclei by 0.25 M sulfuric acid extraction. After incubation of nuclei at 4°C for at least 30 min, the histones were fractionated by centrifugation (supernate fraction) and concentrated by precipitation with 22% trichloroacetic acid.

Labeling of H1 Histones by N-Succinimidyl [3,2-3H]Propionate (NHP): H1 histones were labeled according to a modification of the procedure of Bolt and Hunter (6). N-Succinimidyl [3,2-3H]Propionate (Amer sham Corp., Arlington Heights, Ill., 66 Ci/mmol, 2 µCi/ml) is a succinimide ester that is very stable to aminoo groups. 30-40 µl aliquots of NHP-20 µg of H1 histones in 0.1 M borate buffer (pH 9) were added on ice with occasionally shaking. To retard hydrolysis of the succinimide ester, the reaction mixture volume was kept small (5-30 µl). After 20 min, 100 µl of 0.2 M glycine in 0.1 M borate (pH 9) was added to stop the reaction. The H1 histones were concentrated by precipitation in 22% trichloroacetic acid. 8 x 10⁻⁶ 8 x 10⁻⁶ cpm/µg were routinely incorporated into the histones. The fact that the H1 histones have a higher efficiency of labeling than other proteins tested is probably attributable to the high percentages of lysines in H1.

Cell Electrophoresis

Nuclei Digestions: DNA from nuclease digestion was analyzed on 2.5% agarose slab gels (20 cm long) in the running buffer of Tepper et al. (10). The gels were stained with 1 µg/ml ethidium bromide for at least 30 min, visualized by illumination under ultraviolet light, and photographed through a red filter on polaroid positive-negative film.

H1 Histones: H1 histones were fractionated on 12.5% SDS polyacrylamide gels according to a modification (40) of the method of Laemmli (25). For analysis of peptides generated by proteolysis, 15% SDS polyacrylamide gels were routinely used. Gels were stained with 0.25% Coomassie Blue in 50% methanol and 10% acetic acid and destained by diffusion in 5% methanol and 10% acetic acid.

Protein Mapping: Proteolytic digestion of H1 histones was performed according to the procedure of Cleveland et al. (8). H1 histone bands were cut from 12.5% polyacrylamide gels stained with Coomassie Blue, as previously described, dialyzed in a buffer containing 0.25 M Tris-Cl, pH 6.8, 0.1% SDS, and 1 M glycine, and then applied to the sample wells of 12% polyacrylamide gels. The gel slices were overlaid with Staphylococcus aureus V8 protease (Miles Laboratories Inc., Ames Div., Elkhart, Ind.) in quantities ranging from 3 to 12 µg/slice. Digestion was carried out directly in the stacking gel during the subsequent electrophoresis. Fluorography was performed on the gels to enhance the radioactive detection (7).

Trition Acid-Urea Gel Electrophoresis: Total histones and their subfractions were separated on 12% acrylamide and 0.8% bisacrylamide gels containing 6 mM Triton X-100, 0.9 M acetic acid, and 7.5 M urea (1, 34). Stacking gels containing 6% acrylamide and 0.4% bisacrylamide were used. The gels were preelectrophoresed for 12-20 h. 40 µg of proteamine sulfate (Sigma Chemical Co.) per slot were added and preelectrophoresed an additional 20 min before the samples were added. Gels were stained in 0.25% Coomassie Blue and destained in 5% methanol and 10% acetic acid.

The bands on the triton-acid-urea gels were identified by cutting out each band and electrophoresing on 15% SDS acrylamide gels. Individual protein bands were identified by comparison with known histone standards.

Determination of Fragment Length and Repeat Sizes: Natives of agarose gels showing DNA repeat sizes were stained in a Joyce-Loeb detection system (Joyce, Loeb, and Co., Ltd., Gateshead-on-Tyne, England). Fragment lengths were calculated as described by Morris (29). The size of the DNA fragments were determined by comparison with Hind III digests of SV40 (1725, 1168, 1101, 525, and 446 base pairs). The electrophoretic mobility of the SV40 fragments was plotted against the logarithms of their size. A least-squares regression line was calculated for each of the parent DNA fragments were determined from the mobility of the midpoint of each fragment using the regression formula with the intercept and regression constant derived.

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for the SV40 standards. The repeat lengths were determined by plotting the band number against the fragment size and obtaining the least-squares regression line. The slope of this line gives a value for repeat length that should be independent of "nibbling in" by exonucleases.

The methods of chromosome preparation, staining, and analyses are described in reference 24.

RESULTS

DNA Repeat Lengths in Mouse and Human Cell Lines

To determine whether different rodent and human cell lines have different DNA repeat lengths, we isolated nuclei from six different (three mouse and three human) cell lines and digested them with micrococcal nuclease, usually to 20–30%, acid solubility. The DNA was then purified and separated on 2.5% agarose gels. Because oligomer sizes depend on the amount of nuclease digestion, DNA fragments generated from similar extents of digestion were compared.

Fig. 1 shows the DNA repeat pattern produced by two different mouse lines and one human cell line. HindIII digests of SV40 and adenovirus DNA, as well as nuclease digests of chick erythrocyte nuclei, were coelectrophoresed with the mammalian digests to provide standards for determining numerical repeat sizes of these cells. The nucleosome repeat sizes were measured by determining the slope of the number of base pairs as a function of base number (see Materials and Methods). The DNA band sizes were determined by comparison with a linear regression analysis of the logarithms of the HindIII restriction fragments of SV40 as a function of electrophoretic mobility. The repeat lengths thus determined are 191 base pairs (bp) for mouse line PG19, 187 hp for B82 mouse line, and 183 bp for human cells.

Digestion of mixtures of nuclei from any two of the lines in the same tube did not affect the individual repeat patterns. Densitometer tracings of the gel are shown in Fig. 1 b. Measurement of the migration distance of each sample correlates with the results observed visually. Prolonged nuclease digestions resulted in DNA fragments 140 bp in length in all the populations tested, indicating structural similarities in the core nucleosomes.

Therefore, the difference in repeat lengths is presumably in the linker DNA region. We conclude that the DNA repeat lengths of these three cell lines are distinct from one another. Different degrees of digestion did not affect the repeat sizes of the various cell lines.

To determine the sensitivity of the detection system, we mixed DNA digests from mouse and human cells in proportions ranging from 90:10 to 50:50 and electrophoresed these digests on an agarose gel. The results are presented in Fig. 2. Both visual examination and densitometer tracings of the gels indicate that as little as 10% of the human repeat length can be detected in the presence of 90% of the repeat length of the mouse (cf. slot D and slots C and E). We have further analyzed the repeat lengths by linear regression analysis, as described in Materials and Methods. The results are presented in Fig. 2 c and Table I. The mixture of as little as 10% human and 90% mouse DNA can be very clearly distinguished from the mouse and human repeats (Fig. 2 c). Similar analyses were conducted in determining the DNA repeat sizes of cell hybrids.

Comparison of Hybrids and their Parental Lines

To determine how genomic interactions affect the DNA repeat lengths, we have tested a number of intra- and interspecific cell hybrids. Interspecies hybrid series DUA, PEP, MGM, and BCH are generated from fusion of mouse A9, PG19, or B82 cells with human fibroblasts. PGB series are the result of fusion of mouse B82 with mouse PG19 cells. The mouse x human hybrids retain the mouse genome, whereas only partial genetic complements of the human are present. There is no appreciable degree of chromosome segregation in the mouse x mouse hybrids.

The nuclei from each hybrid were isolated, digested with micrococcal nuclease, and the purified DNA fragments were separated on agarose gels. Comparison of nuclease digests from 24 independent hybrid cell lines obtained from three different mouse x human fusions were made, and representative results are shown in Fig. 3. The repeat sizes of all human lines tested (GM1429, HT1080, and HeLa) are identical, whereas the mouse parental lines PG19, B82, and A9 have distinct repeat lengths. The series of DNA fragments generated by micrococcal nuclease digestions in the BCH hybrid clones are shown in Fig. 3 a. The DNA repeat lengths of all the hybrids are virtually identical with one another and with the mouse parental B82 cells.

In contrast, they fall progressively out of phase at higher multimers with the DNA bands derived from the human parental cells, indicating that the hybrid repeat lengths are different from those of the human cell lines. Similarly, the DNA repeat lengths of PEP and MGM hybrids are all identical to their respective mouse parental lines (Fig. 3 b), implying the dominance of the mouse parental type of nucleosome repeat size in the hybrids. These results are summarized in Table II.

In the intraspecies mouse x mouse hybrids (PGB), agarose gels of the nuclease digest from these hybrids exhibit exclusively the repeat pattern of PG19 cells (Fig. 3 c). Thus, in both classes of hybrids, all chromosomes, irrespective of their cellular origin, acquire a specific DNA repeat length characteristic of only one of the parents.

FIGURE 1 Comparison of micrococcal nuclease digests of nuclei from mouse and human cell lines. (a) Nuclei from two mouse cell lines and one human cell line were digested with micrococcal nuclease and the DNA was extracted and electrophoresed on 2.5% agarose gel (see Materials and Methods). DNA from a micrococcal nuclease digest of chick erythrocyte nuclei, HindIII digests of adenovirus 2 and SV40 DNA were run as standards. The gel was stained with ethidium bromide and photographed under ultraviolet light. (b) Densitometer scans of the micrococcal nuclease digests from the mouse and human cells (a) are shown. The arrows designate the location of the pentamer migration in each of the cell lines. The positions of restriction fragments from a HindIII digest of SV40 that was coelectrophoresed on the same gel shows the following sizes (in base pairs): A, 446; B, 525, C, 1,101; D, 1,168; E, 1,765.
FIGURE 2 DNA repeat lengths in mixtures of mouse and human micrococcal nuclease digests. (a) DNA from mouse PG19 and human HT1080 cells was extracted from nuclei digested with micrococcal nuclease. The isolated DNA from each cell line was mixed in various proportions and electrophoresed on a 2.5% agarose gel (see Materials and Methods). The DNA was stained with ethidium bromide. The slots A–E contained DNA from human and mouse cells in the following ratios: A, 100:0; B, 20:80; C, 0:100; D, 10:90; E, 100:0. (b) Densitometer tracings of slots C, D, and E. Arrows indicate location of pentamer. Migration of HindIII digests of SV40 DNA are shown at the bottom. (see Fig. 1b). (c) Comparison of DNA repeat lengths of tetramers and pentamers of PG19, HT1080, and 90 PG19:10 HT1080 mixture. C, HT1080; PG19; A, 90 PG19:10 HT1080.

TABLE I Migration of DNA from Micrococcal Nuclease Digests of Chromatin from Mouse and Human Mixtures

| Proportion of mouse: human chromatin | Pentamer | Hexamer |
|-------------------------------------|----------|---------|
| 100:0                               | 60.30    | 52.50   |
| 90:10                               | 60.50    | 52.80   |
| 80:20                               | 60.55    | 53.00   |
| 65:35                               | 60.61    | 53.34   |
| 50:50                               | 61.46    | 53.35   |
| 0:100                               | 61.70    | 53.70   |

* Distances obtained from linear regression analyses (see Materials and Methods).

Chromosomal Constitution of Hybrid Cell Lines

To determine the possible role of specific chromosomes in regulating DNA repeat length, we have analyzed the chromosomal constitutions of parental and many of the hybrid cell lines. The mouse x human hybrids retain the mouse genome and segregate human chromosomes. A representative cell from one of the hybrids is shown in Fig. 4. The mouse x mouse hybrids retain essentially all of the chromosomes from both parents. The chromosomal components of the various mouse x human cell lines are presented in Table III. Each of the human chromosomes is represented in at least one of the hybrid cell lines. These results indicate that the presence or absence of any specific chromosome does not affect the DNA repeat length in these hybrids.

Characterization of H1 Histone Subfractions in the Parental and Hybrid Cells

To investigate the relationship between DNA repeat lengths and the H1 expression in a number of cell populations, we attempted to characterize the nature of H1 in parental and hybrid cells. H1 histones were isolated by the extraction of chromatin with 5% perchloric acid. H1 is soluble in perchloric acid, whereas the other histones are not. Electrophoresis of these proteins on 12.5% SDS polyacrylamide gels revealed three H1 histone subfractions (Fig. 5). This same pattern was obtained for all the mouse and human parental cells tested. In an attempt to differentiate the H1 subfractions in different cell lines, we have conducted the following experiments. H1 histones isolated by the above method were labeled in vitro with
TABLE II

Summary of the Nature and Characteristics of Hybrid Cell Lines

| Cell lines | No. of hybrids analyzed | Chromosomes segregating | Average repeat lengths* | Repeat length expressed in hybrids | H1 expressed in hybrids |
|------------|-------------------------|-------------------------|-------------------------|------------------------------------|------------------------|
| A. Mouse x human |                         |                         |                         |                                    |                        |
| PG19 x BP |                         |                         |                         |                                    |                        |
| PG19 |                         |                          |                          |                                    |                        |
| BP |                         |                          |                          |                                    |                        |
| PEP | 9                       | Human                    | 191                     | Mouse                              | Mouse                  |
| PG19 x GM1429 |                             |                         |                          |                                    |                        |
| PG19 |                         |                          |                          |                                    |                        |
| GM1429 |                         |                          |                          |                                    |                        |
| MGM |                         |                          |                          |                                    |                        |
| B82 x HT1080 |                             |                         |                          |                                    |                        |
| B82 |                         |                          |                          |                                    |                        |
| HT1080 |                         |                          |                          |                                    |                        |
| BCH | 5                       | Human                    | 187                     | Mouse                              | NT§                    |
| A9 x DUV |                         |                          |                          |                                    |                        |
| A9 |                         |                          |                          |                                    |                        |
| DUV |                         |                          |                          |                                    |                        |
| DUA | 8                       | Human                    | 200                     | Mouse                              | NT                     |
| B. Mouse x mouse |                         |                         |                         |                                    |                        |
| PG19 x B82 |                         |                         |                         |                                    |                        |
| PG19 |                         |                          |                          |                                    |                        |
| B82 |                         |                          |                          |                                    |                        |
| PGB | 5                       | Retention of both parents | 192                     | PG19                              | B82§                   |

* Repeat lengths were determined by linear regression analysis as described in Materials and Methods.

† NT, not tested

§ The presence of PG19 histone H1 cannot be ruled out (see text).

$^{3}$H-SP (see Materials and Methods). The labeled proteins were separated on a 12.5% polyacrylamide gel. The three H1 histone bands were then sliced and rerun on another 12.5% polyacrylamide gel. Each of the three histone subfractions is clearly separable from the others (Fig. 5) and migrates to the equivalent position in total unfractionated H1 histones.

Each of the H1 histone subfractions from different cell lines was subjected to one-dimensional peptide mapping by proteolysis of gel slices containing different H1 fractions in SDS, with subsequent electrophoresis (8). Representative results of these experiments with Staphylococcus aureus V8 protease are shown in Fig. 6. The peptide patterns generated by this method produced reproducible bands, characteristic of specific H1 subfractions. Furthermore, we were able to distinguish each of the subfractions between species. The peptide maps of H1 subfraction A (Fig. 6a) shows two major bands in the human line that are missing in PG19 mouse line (indicated by arrows). The peptide maps of H1 subfractions B (Fig. 6a) revealed the presence of one band in human cells that is absent in mouse cells. Thus, the patterns of peptides from H1 variants A and B in PG19 cells can be distinguished from those of human origin. Similarly, PG19 mouse line differs from B82 mouse line in both H1 A and H1 B subfractions (Fig. 6b).

Peptide maps generated by protease digestion of H1 subfractions in the mouse x human hybrids (PEP and MGM) and mouse x mouse hybrids (PGB) were examined. The results of some of these experiments are illustrated in the fluorograms in Fig. 6. In all these mouse x human hybrids, the peptide maps of H1 subfractions A and B exhibit a pattern similar to the peptide map of mouse line, PG19 (Fig. 6a). In all of the 11 PEP and MGM hybrid cell lines tested, only the histone H1 of the parental mouse line is expressed. No human H1 was detectable.

Characterization of Histones on Triton Gels

To confirm these observations, we attempted to distinguish species-specific histones in polyacrylamide gels containing Triton X-100, acetic acid, and urea. Acid-extracted histones from parental and hybrid cell lines were subjected to electrophoresis. The nature of the individual bands observed in these gels was established by reelectrophoresis of individual bands in SDS polyacrylamide gels. The general patterns of mobility of several components of mouse and human cell lines are different (Fig. 7). Specifically, there are clearcut differences in the mobility of H2b proteins of rodent and human origin. The patterns of H1 are also different, though not as clear as those of H2b. Examination of the hybrids revealed that all mouse x human hybrids tested have the mouse-specific H2b and H1. Human forms of H2b were not detectable. It was not possible to unambiguously determine the presence or absence of human H1 in these hybrids by this method. The results from both the partial peptide analysis and the triton-acid-urea gels indicate that in these cell hybrids the mouse forms of histones are expressed, to the exclusion of the human forms.

Chromosomal Composition of Hybrid Cell Lines

Because the absence of human H1 expression in these hybrids might be caused by loss of the structural genes, we have analyzed the human chromosomal composition of these cell lines by quinacrine banding techniques (Table III). Each of the individual human chromosomes is represented at least once in these hybrids, implying that the lack of H1 and H2b expression is probably not caused by the absence of the respective structural genes. In the intraspecies hybrids, the peptide maps of the histone subfractions A and B are comparable to the peptide pattern generated by mouse parental line B82 (Fig. 6b). Be-
cause the peptide maps of both H1 A and H1 B of PG19 can be superimposed on the patterns of B82 cells, the presence of H1 histones from PG19 cells, in these hybrids, cannot be ruled out.

DISCUSSION

Nuclease Digestion Studies

Micrococcal nuclease was used as a probe to study the chromatin structure of a number of somatic cell hybrids. We first tested three mouse and three human cell lines and showed that the repeat lengths of the three human cell lines are identical with one another but distinguishable from each of the three different mouse cell lines. That different species (rodent and human) have different repeat lengths is in accordance with earlier observations (10, 27, 29, 32, 37, 40). Direct visual observations, densitometer tracings, and the subsequent determination of numerical repeat lengths of the different cell lines.
yielded consistent results. Subsequent analysis of the hybrids tested indicated the presence of the DNA repeat length of only one parental cell line is expressed. The expression of only one parental type of DNA repeat could be because of (a) our inability to detect one of the repeat lengths in a mixture, (b) segregation of a specific set of chromosomal determinants, or (c) inhibition of the expression of one of the parental phenotypes. We have shown that it is possible to detect the nucleosome repeat length of a parental line whose DNA constitutes as little as 0.01% of the total DNA (Fig. 2). Because the relative proportion of human chromosomes in a number of mouse × human hybrids exceeds 10% (24) and because there is virtually no loss of chromosomes in the mouse × mouse hybrids, failure to detect the DNA repeat lengths is not likely. This evidence, combined with the chromosomal analyses showing the presence of every human chromosome in at least one mouse × human hybrid, suggests that the presence of only one repeat length in all the populations tested cannot be attributed to chromosomal segregation but rather to “dominance” of one of the parental types. From the chromosome analysis, however, we cannot rule out the possibility that two or more specific human chromosomes must be concurrently present for the human phenotype to be expressed, but the results from the mouse × mouse hybrids, in which dominance of one repeat size was observed, indicates that this is unlikely. Because a considerable number of cell generations had elapsed between the cell fusion and our analysis of the hybrids, it was not possible to determine when the DNA of one of the parental types acquires the other repeat pattern.

FIGURE 5 Separation of H1 histone subfractions on an SDS polyacrylamide gel. H1 histones were extracted from nuclei of mouse PG19 cells with perchloric acid. The H1 histone variants were separated on a SDS polyacrylamide gel and stained with Coomassie Blue. H1 histone subfractions A, B, and C were sliced from the gel and rerun on a polyacrylamide gel (slots 2, 3, and 4). Slot 1, total H1 histone subfractions.

TABLE III
Human Chromosomal Composition in 21 Independently Derived Mouse-Human Hybrids *

| Human chromosome | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PG19 × BP‡       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PEP3d            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PEP6a            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PEP7d            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PEP8a            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PEP9c            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PEP12a           | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PEP12b           | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PEP12e           | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| MGM19A           | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| MGM38B           | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| BM1 × GM1429§    | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| BCH3             | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| BCH5             | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| BCH7             | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| A9 × DUV§        | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DUA2b            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DUA4b            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DUA6b            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DUA8a            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DUA9a            | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DUA11a           | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DUA16b           | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DUA20a           | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

* Only those chromosomes present in >10% of the cell populations tested were included.
‡ The experimental procedure and characteristics of these hybrids are described in reference 24.
§ These results are compiled from human-marker analysis and do not include information regarding chromosomes 3, 4, 9, 17, and 22 (35).
¶ Chromosome present in 3-7% of cells.
FIGURE 6 Comparison of partial peptide maps of H1 histone subfractions from mouse x human and mouse x mouse hybrid cell lines. Labeled H1 histone subfractions from different parental mouse and human lines and their hybrid clones were separated on SDS polyacrylamide gels. The individual subfractions were isolated and subjected to proteolysis by Staphylococcus aureus protease followed by electrophoresis on SDS polyacrylamide gels. (a) Partial peptide maps of H1 subfractions A and B in PEP and MGM hybrids. Arrows indicate bands present in the human parental cells but absent in the mouse or hybrid cells. (b) Partial peptide maps of H1 fractions A and B in PGB (mouse x mouse) hybrids. Arrows indicate partial peptides present in B82 and hybrid cells but not in PG19.

H1 Histone Analysis

H1 histone subfractions of different parental and hybrid cell lines were analyzed by peptide mapping (8). Partial peptide maps were generated from labeled H1 histone subfractions A, B, and C. We have compared the patterns produced by digestion with trypsin, chymotrypsin (results not shown), and S. aureus protease. Detectable differences in the band patterns of subfractions A and B were observed after proteolysis with S. aureus protease. The peptide band patterns that are obtained from each cell line were consistent, reproducible, and were detectable after various degrees of digestion. Comparison of the peptide maps of the different subfractions of H1 indicate that H1B is most likely a unique protein rather than a proteolytic product or modified H1A. Although common peptides between various cell lines exist, clearly identifiable differences were observed among several mouse and human cell lines. It is possible that this method might be useful in distinguishing between specific forms of the other histones (H2a, H2b, H3, and H4).

Peptide mapping was performed on inter- and intraspecific hybrids. The partial peptide maps of H1 subfractions of B82 and PG19 mouse lines are distinguishable. In all the intraspecific PG19 x B82 hybrids tested, we detected the presence of the H1 histones of B82 mouse cells. The presence of H1 histone from the other parental cells (PG19) cannot be eliminated because the peptide maps of both H1 subfractions A and B of PG19 cells can be superimposed on the histone patterns of B82 cells.

In the mouse x human hybrids (PEP and MGM), we have observed only the mouse form of H1A and H1B (Fig. 6a). Because human H1 A and H1 B exhibit bands not present in the peptide maps of the mouse cells, it is unlikely that the human H1 histones are expressed and not detected.

The results from the partial peptide analysis of histone H1 were extended by Triton-acid-urea gel analyses (Fig. 7). This method enables the detection of single charge differences in these proteins (34). We have observed that the H2b and possibly the H1 histones of human origin have electrophoretic patterns that are distinguishable from the corresponding mouse forms. The different forms of these proteins observed in these gels could reflect either differences in amino acid composition
or posttranslational modifications. The reproducibility of the electrophoretic patterns and relative quantities of the H2b subfractions is consistent with differences in amino acid composition, though other explanations cannot be completely ruled out. The results from the two distinct methods we have employed to detect differences in histone gene expression indicate that the expression of both H1 and H2b of human origin is suppressed in cell hybrids. It is likely that the expression of other human histone genes is similarly affected.

On the basis of the chromosome analysis, it is unlikely that the lack of expression of the human H1 gene(s) is caused by the loss of a specific human chromosome (presumably containing the structural gene(s)). Each of the 23 different human chromosomes is present at least once in the population of hybrids tested (Table III); in addition, the frequency of retention of each of the human chromosomes exceeded 30%.

Studies on the sequence organization of histone genes in sea urchins (21, 22, 38, 39) and Drosophila melanogaster (26) have demonstrated that histone genes are reiterated and arranged in a unit containing the genes for all 5 histones interspersed with noncoding spacer sequences. Yu et al. (43) reported the localization of human H4 histone genes on chromosome 7 by in situ hybridization. There is a strong possibility that the sequence organization of human histone genes is similar to that found in other eukaryotes. If this is the case, the genes coding for the other histones are probably located on human chromosome 7.

A number of the mouse × human hybrids we have tested contained chromosome 7 at frequencies up to 85%.

Because the human H1 histones are absent in these hybrids, the human DNA is presumably able to associate with the mouse H1 histone in a biologically functional manner. The individual chromosome banding patterns, as revealed by various techniques, are not altered in hybrids and both parental genomes retain their capability to express their genes (24). The association of mouse H1 histones with the chromatin of another species in an active conformation is consistent with results reported by Hohmann et al. (20). Though our results deal with H1, it is possible that all other human histones (H2a, H2b, H3, and H4) are similarly affected. Indeed, Ajiro et al. (2) have shown species-specific suppression of H1 and H2b production in mouse × human hybrids. Elicieri and Green (12), and Marshall et al. (28) have observed that human ribosomal genes, though retained, are not expressed in mouse × human hybrids that are segregating human chromosomes. The observations regarding H1 expression presented in this report provide a parallel situation. The nature of the mechanism for this differential expression of genes coding for structural components and its possible significance are not clear.

Relationship of H1 and Repeat Length

A number of studies have implied a causal relationship between H1 histone and the chromatin repeat lengths (29, 32, 37). The fact that all the mouse × human hybrids we have tested have the repeat lengths and H1 of the mouse type is consistent with this notion. Because of the lack of extensive chromosome segregation, the mouse × mouse hybrids are more informative in this regard. If there is a strict correlation between H1 histones and DNA repeat length, we would expect to find one of the following situations in PGB hybrids: (a) H1 histones and DNA repeat lengths of B82 cells, (b) H1 histones and DNA repeat lengths of PG19 cells, or (c) a mixture of both. We have, however, observed that the DNA repeat pattern is exclusively that of PG19 cells, whereas the H1 histones of B82 cells are certainly expressed. If these results are confirmed in other interspecific hybrid combinations involving parents with more distinct repeat lengths, it will indicate that if H1 histones play a role in determining nucleosome repeat size, they might do so in concert with one or several nonhistone chromosomal proteins, and not exclusively by themselves.

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