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Genetics

Species-specific suppression of histone H1 and H2B production in human/mouse hybrids

(cell regulation/histone genes/chromosome segregation/human fibrosarcoma)

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ABSTRACT **Ten human/mouse hybrid cell lines that segregate either human or mouse chromosomes were examined for the expression of human- and mouse-specific histones H1 and H2B. Results of this study indicate that the human and mouse chromosomes in hybrid cells that segregate human chromosomes (M > H hybrids) contain only mouse histones H1 and H2B. Chromosomes in hybrid cells that segregate mouse chromosomes (H > M hybrids) contain only human H1 and H2B histones. Loss of the ability to produce either human or mouse histones does not seem to be due to the loss of specific human or mouse chromosomes because M > H hybrids retaining at least one copy of each human chromosome contain only mouse H1 and H2B and H > M hybrids retaining at least one copy of each mouse chromosome contain only human H1 and H2B histones. These results, together with those concerning histone H4 acetylation levels and ratios of variants of histones H3 and H2A that are like those in the dominant parent cell type, indicate that the control mechanisms affecting H1 and H2B expression in H > M and in M > H hybrid cells affect expression of histones H2A, H3, and H4 genes as well. The present data thus support the hypothesis that none of the histone genes that are active in the recessive parent cell type is expressed in hybrid lines that segregate recessive cell chromosomes.**

Croce *et al.* (1, 2) have shown that human/mouse somatic cell hybrids segregating human chromosomes (M > H) produce only mouse 28S ribosomal RNA even when they retain copies of the human chromosomes that contain the genes for 28S ribosomal RNA. Similarly, somatic cell hybrids that segregate mouse chromosomes (H > M) produce only human 28S ribosomal RNA even when they retain copies of mouse chromosomes that contain the 28S ribosomal RNA genes. Recently, Perry *et al.* (3) studied these human/mouse hybrids for the expression of mouse and human 45S ribosomal RNA precursors and for the presence of mouse and human ribosomal genes. They concluded that a selective suppression of the transcription of either human or mouse ribosomal genes occurs in the hybrid cells. An interesting phenomenon that parallels the species-specific suppression of ribosomal RNA production is the suppression of the replication of small DNA tumor viruses for which the recessive species is normally permissive (4). Because it has recently been shown that it is possible to distinguish mouse and human H1 and H2B histones (5, 6), we examined the expression of human and mouse histones in human/mouse somatic cell hybrids to determine whether mouse and human histones are coexpressed in the hybrid cell or whether a similar phenomenon of species-specific suppression occurs in the hybrids.

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MATERIALS AND METHODS

Cells. HT1080 human diploid fibrosarcoma cells were hybridized to mouse peritoneal macrophages (hybrids 55-14F1, 55-14F7, 55-91F1, 55-54F2, and 55-54F4), to cells derived from the solid murine OTT6050 teratocarcinoma (hybrids 55-84F4 and 55-84F8), and to cells derived from a continuously growing mouse cell line (THO2) (hybrids 56-05F2, 56-05F4, and 65-05F5), according to established procedures (7). Because it has been reported that the genes for human histone H4 are located on human chromosome 7 (8), we included in this study a mouse/human hybrid line (Nu 9) segregating human chromosomes but containing human chromosome 7 in 100% of its cells (9). The results of karyotype analysis of these hybrids have been reported (1–4). In hybrids of the 55-84 series, every mouse chromosome was present (1, 3, 4). The results of karyotype analysis were confirmed by isozyme analysis for markers assigned to each of the human chromosomes and to 14 different mouse chromosomes. HeLa cells are human carcinoma cells and L cells are mouse cancer cells derived from a C3H mouse.

Histone Analysis. Cells were scraped from glass culture bottles in buffer A [10 mM K Tris·maleate, pH 7.4/50 mM glycine/5 mM $MgCl_2$/1% thiodiglycol (Pierce)/0.1 mM N^{α}-tosyllysyl chloromethyl ketone (TLCK)/0.1 mM *N*-tosylphenylalanine chloromethyl ketone (TPCK)] and then homogenized in a Dounce homogenizer until at least 90% of the cells were broken. The nuclei were sedimented through 1/3 vol of buffer A containing 5% sucrose at 200 × *g* for 5 min. They were washed successively with buffer A containing 1% Triton X-100 and then with buffer A containing 2 mM $CaCl_2$. The nuclei were suspended in 10 vol of buffer A lacking divalent cations but containing 50 units of micrococcal nuclease (Sigma) per ml. After resuspension, the nuclei were frozen at −70°C, thawed after the addition of 1/4 vol of 5 M NaCl and 0.5 mg of protamine, dispersed in a micro-Dounce homogenizer, and centrifuged at 10,000 × *g* for 10 min to remove insoluble material. The nucleic acids and acid-insoluble proteins were precipitated by the addition of HCl to 0.2 M and removed by centrifugation at 10,000 × *g* for 10 min.

The histones were recovered by precipitation with 20% (wt/vol) trichloroacetic acid, washed with acetone containing 1% thiodiglycol and 0.2% HCl, washed again with acetone plus 1% thiodiglycol, dried under reduced pressure, and dissolved in 100 μl of 8 M urea/5% mercaptoethanol/1% thiodiglycol. Samples containing 30–40 μg of the histones were then resolved by electrophoresis on 30-cm-long 12% polyacrylamide gels

Abbreviations: M > H, human/mouse somatic cell hybrids segregating human chromosomes; H > M, human/mouse somatic cell hybrids segregating mouse chromosomes; TLCK, N^{α}-tosyllysine chloromethyl ketone; TPCK, *N*-tosylphenylalanine chloromethyl ketone.

containing 5% acetic acid and 6 mM Triton X-100, according to Zweidler (10). The gels were stained with Amido Black, destained by diffusion, and scanned at 300 nm. The ratios of variants within a given histone class were obtained from those electrophoretic conditions that gave the best resolution for each class. Thus, H2A variants were analyzed in 7.5 M urea, H3 variants in 6 M urea, and H2B and H1 variants in 3 M urea.

For analyses of histone H1 subtypes, all procedures were carried out at 4°C unless otherwise indicated. Cellular monolayers containing $\approx 2 \times 10^7$ cells per culture flask were washed three times with spinner salts (11) and then removed with a policeman in 25 ml of 80 mM NaCl/20 mM EDTA/1% Triton X-100/0.05 M sodium bisulfate/0.1 mM TLCK/0.1 mM TPCK, pH 7.4. After being washed twice in 25 ml of this solution by centrifugation at 600 × *g* for 5 min, saline-soluble proteins were removed by washing twice in 0.15 M NaCl/0.05 M sodium bisulfate/0.1 mM TLCK/0.1 mM TPCK. Histones were extracted twice from the resultant nuclear pellets in 200 μl of 0.2 M H_2SO_4, and the pooled extracts were precipitated overnight at −20°C with 4 vol of ethanol. After being harvested by centrifugation at 10,000 × *g* for 10 min, the histone pellets were dissolved in 20–50 μl of deionized water, and the amount of protein present in each sample was estimated spectrophotometrically (1 mg = 7 A_{218}).

To isolate the histone H1 fraction, we mixed acid-soluble nuclear protein extracts dissolved in water with an equal volume of 10% (vol/vol) perchloric acid and allowed them to precipitate for 60 min on ice. Nucleosomal core histones were harvested by centrifugation at 10,000 × *g* for 10 min; the supernatant containing H1 histones was removed and the pellet was reextracted twice with 200 μl of 5% perchloric acid. After the pooling of all of the 5% perchloric acid extracts, H1 was precipitated by the addition of 1.2 g of trichloroacetic acid per ml and chilling on ice for 60 min. H1 was pelleted by centrifugation at 10,000 × *g* for 10 min, washed once in acetone/0.3% HCl and twice in acetone, and then resuspended in a minimal volume of water. Samples containing 5–10 μg H1 were resolved by electrophoresis at 60 V for 70 hr on slab gels containing 12% acrylamide and 1% sodium dodecyl sulfate (12) in a Hoeffer SE-500 apparatus. After electrophoresis, the gels were soaked for 3–4 hr in methanol/acetic acid/water, 2:1:7 (vol/vol), and then stained for 60 min in a similar solution containing 0.2% Coomassie blue. After electrical destaining (13), the gels were scanned at 600 nm in a Corning 750 densitometer.

Table 1. Ratios of histone variants and histone H4 acetylation levels in human/mouse hybrids and in their parent cell lines

Cell line	Principal histone types				
	H2A	H2B	H3	H4	H1
	2A.1 / 2A.2	2B.1 / 2B.2	3.2 / 3.1	OAC / AC	H1 / H1°
HT1080 (human parent)	1.20	21.63	1.224	2.21	14.11
THO2 (mouse parent)	1.44	0.98	3.158	1.04	3.50
Hybrid 55-84F8 (H > M)	1.00	28.97	1.278	1.35	15.72
Hybrid 56-05F4 (M > H)	1.33	0.65	2.814	0.88	4.79
HeLa	1.55	22.66	0.82	2.51	9.33
L cells	1.23	1.59	3.33	1.43	13.52

H1°, H1-related histone usually associated with nondividing cells.

RESULTS

It can be seen in Fig. 1 that there are species-specific (6) qualitative differences between the H2B histones of the human HT1080 and mouse THO2 cell lines that served as parents of interspecific cell hybrids examined in this study. When their histones were resolved by electrophoresis on polyacrylamide gels containing acetic acid, urea, and Triton X-100, HT1080 human fibrosarcoma cells, like other human cells, contained only one principal type of histone H2B (H2B variant 1, H2B.1). In contrast, mouse THO2 cells contained nearly equimolar amounts of H2B.1 and H2B.2 (Table 1). Previous studies (6) have shown that, except for glycyl residue at position 75 in H2B.1 and a seryl residue at the same position in H2B.2, these two mouse H2B histone variants have identical amino acid sequences. A more detailed analysis of the histones of these human and mouse parent cell types, shown in Figs. 2 and 3 and summarized in Table 1, indicates species-specific quantitative differences in the relative amounts of the three histone H3 variants (6) in the cells, in H4 acetylation levels, and in the relative amounts of histones H1 and H1° (H1° is H1-related histone usually associated with nondividing cells).

It is evident from Fig. 1 that 55-84F8 (H > M), a hybrid cell line that loses (segregates) mouse chromosomes during prolonged culture but still retains every mouse chromosome, expressed only H2B.1, as does the human HT1080 parent cell. In contrast, 56-05F4 (M > H), a hybrid cell line that segregates human chromosomes but still retains every human chromosome (determined by karyologic and isozyme analysis as described in refs. 1–4) expressed both H2B.1 and H2B.2, as did the THO2 mouse parent cell.

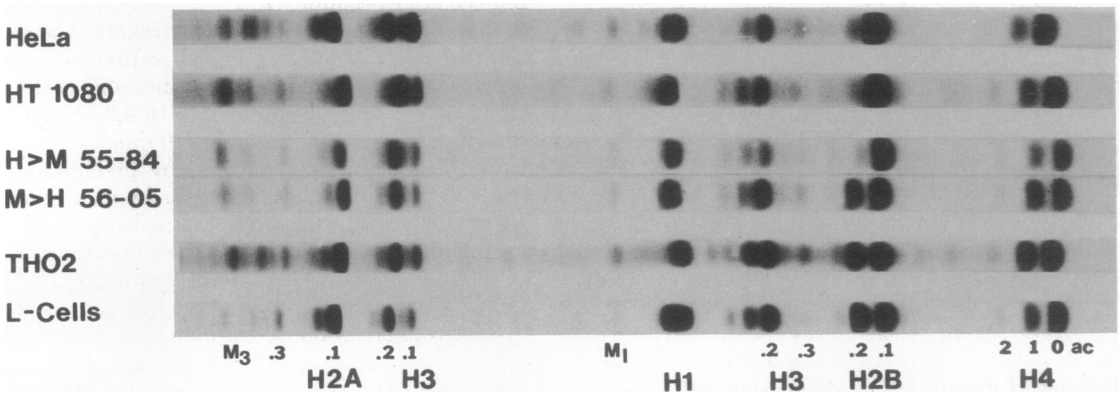

FIG. 1. Amido Black-stained histones of human, mouse, and human/mouse hybrid cells resolved in 30-cm-long 12% polyacrylamide gels containing 5% acetic acid, 7.5 M urea, and 6 mM Triton X-100. Electrophoresis was from left to right. The top 7 cm of the gels is not shown. M1 and M3, minor histone species (14); 0.1, 0.2, and 0.3, primary structure variants of H2A, H3, and H2B (6). Note the difference between human and mouse cells in the presence of H2B.2 as well as the relative amounts of H3.2 and the acetylated forms of H4.

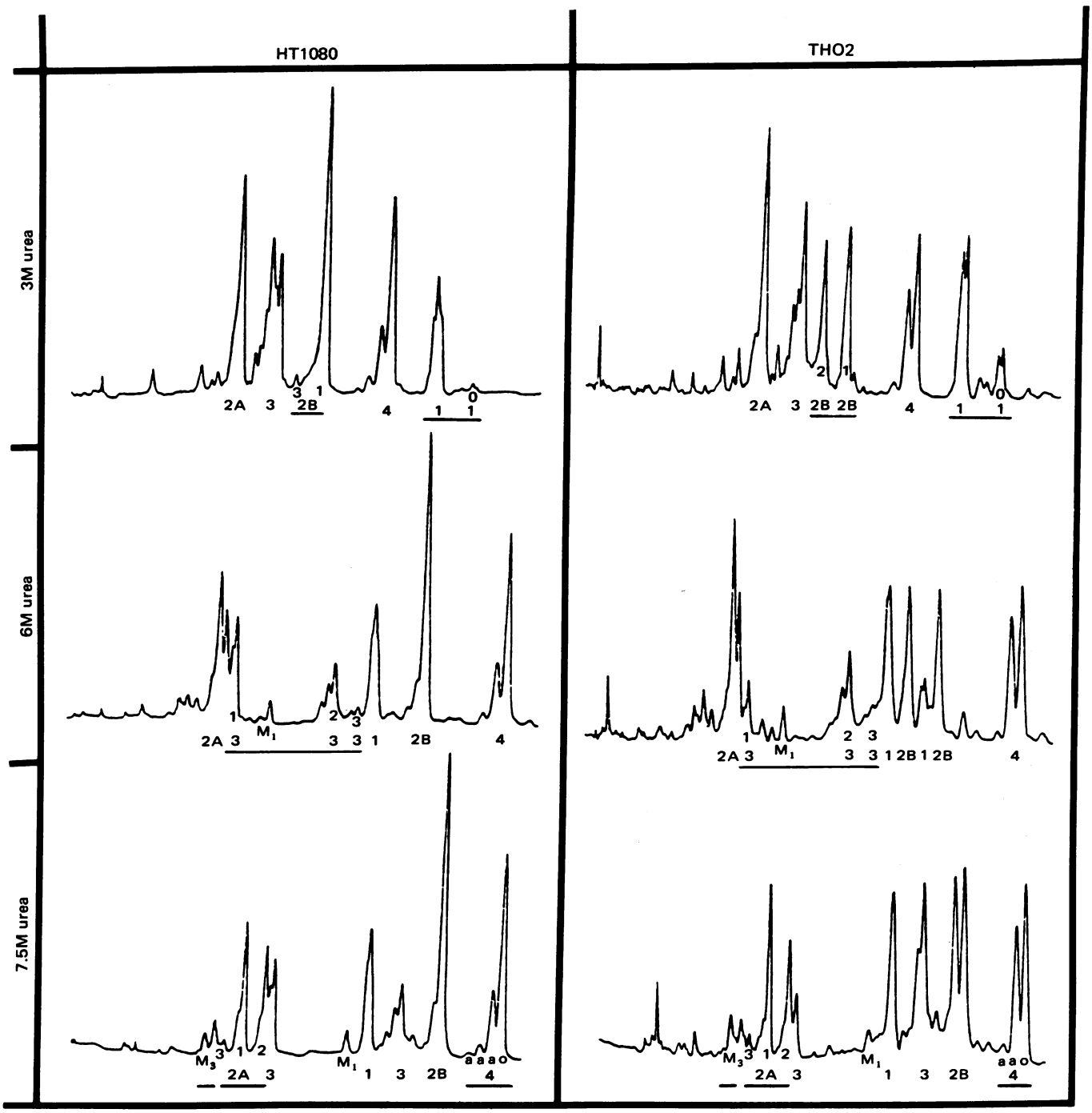

FIG. 2. Spectrophotometric scans of Amido Black-stained histones of the human (HT1080) and mouse (THO2) parent cell histones after electrophoresis in polyacrylamide gels containing 5% acetic acid, 6 mM Triton X-100, and 3, 6, or 7.5 M urea. Note that the different conditions gave optimal resolution for different histones. The regions in each condition used for the quantification of different histone variant ratios, as shown in Fig. 3 and Table 1, are underlined. Nomenclature as in Fig. 1; H1°, H1-related histone usually associated with nondividing cells.

Detailed quantitative analyses of the histones in these two human/mouse hybrid cell lines, shown in Fig. 3 and summarized in Table 1, clearly indicate that species-specific characteristics of only the dominant parent cell line are expressed. Thus, the mouse chromosome-dominated hybrid 56-05F4 contained about equal amounts of H2B variants 1 and 2 whereas the human chromosome-dominated hybrid 55-84F8 showed only a small amount of material in the position of H2B.2 [probably corresponding to the H2B variant 3 described in calf thymus (6)]. That this suppression may be a general phenomenon affecting all recessive cell histone genes is indicated by the H3.2/H3.1, H2A.2/H2A.1, and H1/H1° ratios, which have values in both hybrid lines quite close to those of the dominant parent cell type rather than intermediate values. Although histone acetylation level comparisons are complicated by the fact that acetylation is dependent on growth rates and acetyl metabolism, it is interesting to note that mouse THO2 parent cells and mouse chromosome-dominated 56-05F4 hybrid cells each have at least 10% more acetylated histone H4 than do human HT1080 parental cells or 55-84F8 (H > M) hybrids. It seems from Fig. 3 and from other studies (unpublished results) that the level of acetylation of histone H4 may be a species-specific characteristic reflecting a difference in the accessibility of H4 acetylation sites in human and mouse nucleosomes.

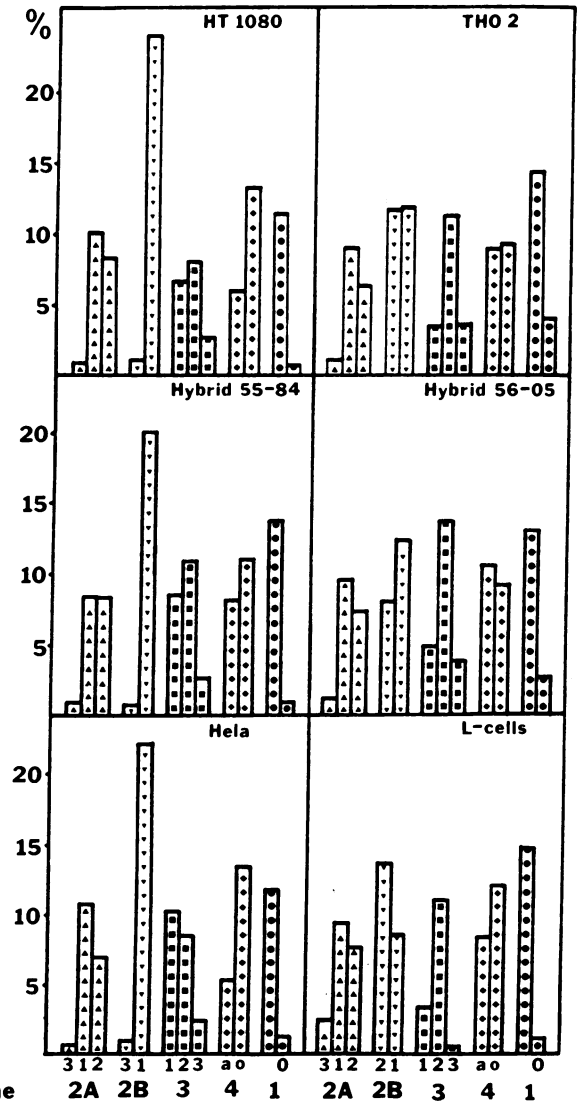

FIG. 3. Relative amounts of different histone components in the human, mouse, and human/mouse hybrid cells shown in Fig. 1. The components were quantified in three different electrophoretic systems, as demonstrated in Fig. 2. Note the striking similarity between the profiles of the H > M hybrid 55-84F8 the human parent HT1080 and of the M > H hybrid 56-05F4 and the mouse parent TH02.

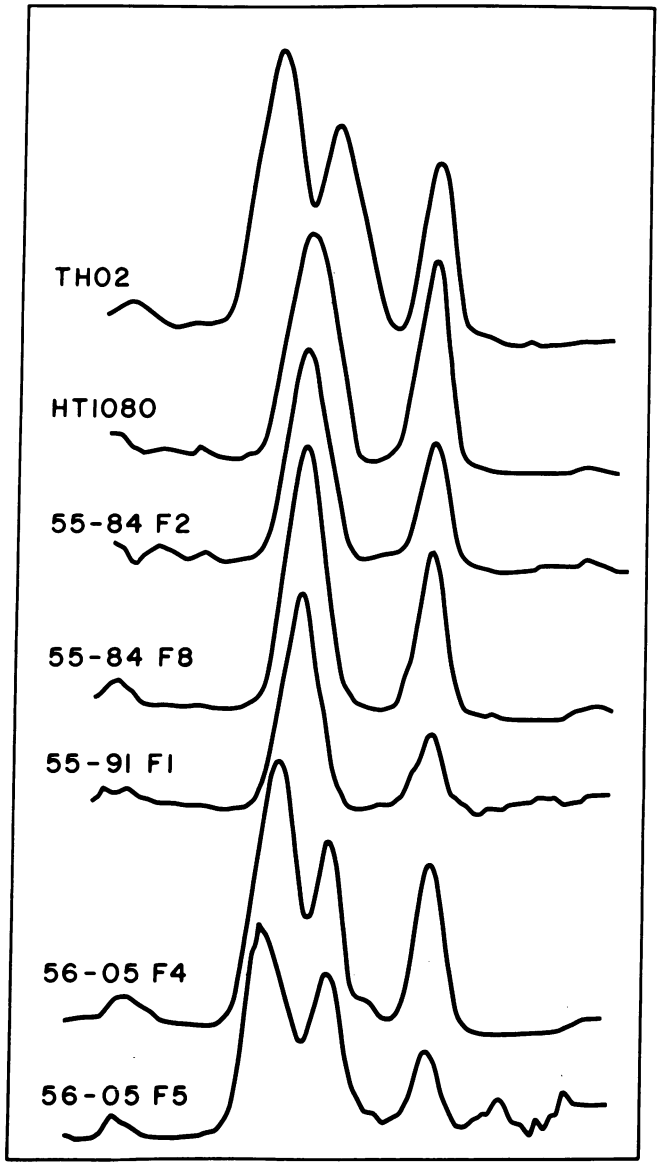

FIG. 4. Densitometric tracings of histone H1 subtypes resolved by electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate. Samples containing 5–10 μg of purified H1 histones were extracted from human/mouse hybrid cells and their parent cell lines and were resolved by electrophoresis at 60 V for 70 hr on 15 × 30 cm polyacrylamide slab gels containing 1% sodium dodecyl sulfate. After the staining and destaining, the regions of the gel containing H1 subtypes other than H1° were scanned at 600 nm in a Corning 750 densitometer. Electrophoresis was from left to right.

Although relative values of H1/H1° ratios (Fig. 3; Table 1) obtained in the preceding analyses suggest that there are species-specific differences between human and mouse histone H1 polypeptides, differences in principal H1 subtypes are more clearly resolved by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (13). It can be seen in Fig. 4 that, under these analytical conditions, the H1 fraction of human HT1080 parent cells contained two principal component subtypes whereas the H1 fraction of mouse THO2 parent cells contained three resolvable subtypes. A comparison of the H1 subtypes in human, mouse, and various human/mouse hybrid cells is also shown in Fig. 4. All H > M hybrid cell lines (55-84F4, 55-84F8, and 55-91F1) expressed the human histone H1 pattern; in contrast, the M > H hybrid lines (56-05F4 and 56-05F5) expressed the mouse H1 pattern. Table 2 summarizes the results of these and similar analyses of different hybrid cell lines for the expression of mouse and human histones H1 and H2B.

DISCUSSION

The results presented in this paper indicate that human/mouse somatic cell hybrids segregating human chromosomes express only mouse histones H2B and H1 and that hybrids segregating mouse chromosomes express only human histones H2B and H1. Loss of the ability to produce the histones of the species whose chromosomes are segregated does not seen to be due to the loss of specific human or mouse chromosomes because the M > H and the H > M hybrid cell lines tested retained every chromosome of the recessive species. These results parallel the findings of the suppression of human and mouse ribosomal RNA in mouse/human hybrids segregating human and mouse chromosomes, respectively (1–3). The suppression of the production of ribosomal RNA also does not appear to be due to the

Table 2. Expression of human (H) and mouse (M) histones in hybrids

Hybrid cell line	Type of hybrid	Histone type H2B	Histone type H1
55-14F1	H > M	H	H
55-14F7	H > M	H	H
55-54F2	H > M	H	H
55-54F4	H > M	H	H
55-84F4	H > M	H	H
55-84F8	H > M	H	H
Nu 9	M > H	M	M
56-05F1	M > H	M	M
56-05F4	M > H	M	M
56-05F5	M > H	M	M

loss of specific chromosomes (1, 2). However, it is not clear at present whether species-specific suppression of the production of histones H2B and H1 occurs at the transcriptional level, as with ribosomal RNA (3), or at some post-transcriptional level of control.

The present results also strongly support the hypothesis that the histones present in the human and mouse chromosomes in H > M hybrids must have been coded for by the human parent genome and that histones in both types of chromosomes in M > H hybrids must have been coded for by the mouse parent genome. Ancillary experiments (data not shown) indicate that, under the analytical conditions used here, contamination of human histones with <5% mouse histones could have been detected in H > M hybrid cell lines, if it existed. It is thus possible that selective segregation of recessive parent cell chromosomes in interspecific hybrid cells could be induced, in part, by the absence of the appropriate species-specific histones during chromosome replication in hybrid cell lines.

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1. Croce, C. M., Talavera, A., Basilico, C. & Miller, O. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 694–697.
2. Miller, O. J., Miller, D. A., Dev, V. G., Tantravahi, R. & Croce, C. M. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4531–4535.
3. Perry, R. P., Kelly, D. E., Schibler, V., Huebner, K. & Croce, C. M., *J. Cell Physiol.*, in press.
4. Huebner, K., Shander, M. & Croce, C. M. (1977) *Cell* **11,** 25–33.
5. Zweidler, A. (1976) *Arch. Genet.* **49,** 94.
6. Franklin, S. & Zweidler, A. (1978) *Nature (London)* **266,** 273–275.
7. Croce, C. M. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3248–3252.
8. Yu, L. C., Szabo, P., Borun, T. W. & Prensky, W. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 1101–1105.
9. Khoury, G. & Croce, C. M. (1975) *Cell* **6,** 535–542.
10. Zweidler, A. (1977) in *Methods in Cell Biology,* ed. Prescott, D. (Academic, New York), Vol. 17, pp. 223–233.
11. Stephens, R. E., Pan, C. J., Ajiro, K., Dolby, T. W. & Borun, T. W. (1977) *J. Biol. Chem.* **252,** 166–172.
12. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
13. Marks, D., Paik, W. K. & Borun, T. W. (1973) *J. Biol. Chem.* **248,** 5660–5667.
14. Zweidler, A. (1976) in *Life Science Research Report (Dahlem Konferenzen),* eds. Allfrey, V. G., Bautz, E. K. S., McCarthy, B. J., Schimke, R. T. & Tissieres, A., Vol. 4, pp. 187–196.