Expression of human and mouse nonhistone chromosomal proteins in hybrid mouse erythroleukemia cells containing a single human chromosome
(chromatin/two-dimensional gel electrophoresis)

U. BODE*, A. DEISSEROTH, AND D. HENDRICK†

Experimental Hematology Section, Pediatric Oncology Branch, Building 10, Room 3B07, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

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ABSTRACT The nonhistone chromosomal proteins of a series of hybrid mouse erythroleukemia cell lines containing human chromosome 16 were investigated by two-dimensional gel electrophoresis to determine if such cells contained nonhistone chromosomal proteins of both human and mouse origin. Comparison of the two-dimensional gel electrophoreograms of the nonhistone chromosomal proteins of mouse and human cell lines showed 400 and 250 chromosomal proteins, respectively, of which about 75% were electrophoretically identical. The two-dimensional gel electrophoreogram of a cloned hybrid mouse erythroleukemia cell line that retained a tetraploid complement of mouse chromosomes and human chromosome 16 (as the only human chromosome) displayed a nonhistone chromosomal protein of pI 6.2 and M, 65,000. This protein, which comigrates with a nonhistone chromosomal protein present in the human cell line used to produce this hybrid cell and which is also present in two additional human cell lines studied, could not be detected in the mouse erythroleukemia parent before fusion. This polypeptide also was shown by similar techniques to be associated with the presence of human chromosome 16 in four of five other independently derived hybrid mouse erythroleukemia cell lines that contained a near tetraploid complement of mouse erythroleukemia chromosomes.

Recent studies have shown that chromosomal proteins are important structural elements of the cell nucleus that may participate in regulating the expression of genes that serve as markers for the differentiated state of tissues (1, 2). Nonhistone chromosomal proteins are a very complex subset of chromosomal proteins displaying apparent species and tissue specificity in cell-free transcriptional systems (3, 4). In this study we investigated the nonhistone chromosomal proteins of human–rodent hybrid cells containing a single human chromosome. It was decided to use this approach to test if a comparison of the chromosomal protein pattern of parental and hybrid cell lines could be used to identify proteins in the hybrid cells that correlate with the presence of specific human chromosomes.

Two groups of workers have reported studies of chromosomal proteins in hybrid cells. Ajero et al. (5) used one-dimensional gel electrophoresis to show that the patterns of the histones H2B were distinct in the mouse and human parents and that hybrids which retain primarily mouse chromosomes express the mouse forms of H2B, whereas hybrids which retain primarily human chromosones contain the human but not the mouse-specific H2B. Lyderson et al. (6) studied the nonhistone chromosomal proteins of a series of human–Chinese hamster hybrids which had retained a majority of Chinese hamster chromosomes (6). Only 10% of the human nonhistone chromosomal proteins were unique from those of the Chinese hamster parent in the two-dimensional electrophoresis system used, and only one of the seven hybrid cells studied contained an electrophoretic species that comigrated with any of the nonhistone chromosomal proteins of the human parent.

Using two-dimensional gel electrophoresis (7), we have identified 400 and 280 nonhistone chromosomal proteins in the mouse and in the human parental cell lines, respectively. Of the 280 nonhistone chromosomal proteins found in the human cells, 70 were not present in the mouse erythroleukemia cells studied. If the genes for these human nonhistone chromosomal proteins were distributed equally among 46 human chromosomes, one would anticipate that the genes for one to two such human specific nonhistone chromosomal proteins would be associated with each human chromosome. In fact, five of the six hybrid cell lines that contained human chromosome 16 did indeed contain a single M, 65,000 nonhistone chromosomal protein of pI 6.2, which was not found in the parent mouse cell but which comigrated with a similar protein found in the human parent cell line used for fusion and in two other human cell lines studied. These studies showed, therefore, that nonhistone chromosomal proteins that are absent in mouse cells and that comigrate with human nonhistone chromosomal proteins can be found in mouse–human hybrid cells containing a majority of mouse chromosomes.

MATERIAL AND METHODS

Cells. Fusion of 1 × 10⁶ 2292 cells and 1 × 10⁷ 179 MEL cells was performed in the presence of 40% (vol/vol) polyethylene glycol and 10% (vol/vol) dimethyl sulfoxide as described (8). The hybrid cell line 323-4 was isolated in F 12 medium containing 0.002 M alanose, 10% (vol/vol) fetal calf serum, 1 μM ouabain, and adenosine as the only purine source (8–11). This selective system is lethal for the human parent and promotes the growth of hybrid cells that have retained human chromosome 16, which contains the human α-globin gene (9) and the adenine phosphoribosyltransferase (APRT; AMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.7) gene (APRT) as reported (10).

Chromosomal analysis and APRT determinations were done as described (11, 12). For the analysis of the chromosomal proteins, a suspension of 150 ml of these cells was incubated at a concentration of 2–5 × 10⁶ cells per ml with 25 μCi (1 Ci = 3.7

Abbreviation: APRT, adenine phosphoribosyltransferase (AMP: pyrophosphate phosphoribosyltransferase, EC 2.4.2.7).

† Present address: Bethesda Research Laboratories, Rockville, MD 20850.
× 10^{10} becquerels) of [35S]methionine (Amersham; specific activity, 1000 Ci/mmol) per ml for 24 hr before harvesting.

Isolation of Chromatin. All procedures were performed at 4°C and in the presence of 0.5 mM diisopropylfluorophosphate (for protease inhibition) and 1 mM dithiothreitol. Cells (150 cc) at the concentration of 2–50 × 10^6 cells per ml were harvested and washed once with serum-free medium. Cells were lysed by washing with 0.5% Triton X-100 containing lysis buffer (0.25 M sucrose/1.5 mM MgCl₂/0.15 M NaCl/10 mM Hepes, pH 8.0). Repeated washings with the nondetergent-containing lysis buffer and with 10 mM Tris/10 mM NaCl/1.5 mM MgCl₂, pH 7.4, were used to obtain a preparation of nuclei. The supernatants were combined as the cytoplasmic fraction.

Detergent solution [10% (wt/vol) Na deoxycholate/10% (wt/vol) Tween 40, 1:2 (vol/vol)] was added 1:12.5 (vol/vol) to the Tris/NaCl/MgCl₂ buffer to disrupt the nuclei. This step was followed by repeated washing with Tris/NaCl/MgCl₂ buffer and 0.075 M NaCl/0.024 M EDTA, pH 8.0. After resuspending the chromatin in 0.05 M Tris (pH 8.0), it was homogenized by 20 strokes in a glass Teflon homogenizer in the presence of 10% (vol/vol) sucrose. The chromatin was purified over a 45–55% sucrose gradient by centrifugation at 135,000 × g for 2 hr. All supernatants were again combined to generate the nucleoplasmic protein fraction.

Protein Isolation. The chromatin pellet was resuspended in 300 μl of a solution of 7 M urea/3 M NaCl/10 mM Tris, pH 8.3, mixed vigorously for about 4 min, and centrifuged at 135,000 × g for 42 hr. The protein supernatant was separated from the pelleted DNA.

The cytoplasmic and nucleoplasmic protein fractions were dialyzed briefly against 10 mM Tris, pH 7.4/0.1% Triton X-100. The volume of the nucleoplasmic fraction was decreased by drying with polyethylene glycol, and then both fractions were dialyzed for 2 hr against 9.2 M urea/1% Triton X-100/10 mM Tris, pH 7.5. Before storage, Ampholines and 2-mercaptoethanol were added to a final concentration of 2% and 5% (vol/vol), respectively.

![Fig. 1. Chromosomal analysis of hybrid cell 323-4 clone 8:20 by (Left) Giemsa/trypsin banding technique. (Right) Subsequent species-specific fluorescence staining with Hoechst dye 33258. Arrow indicates human chromosome 16. All hybrids were characterized by study of at least 25 metaphase spreads by Giemsa staining. An additional 25 metaphase spreads stained by Giemsa/trypsin banding followed by treatment with Hoechst dye 33258 were used to characterize in detail the composition of human chromosomes in selected hybrid cells as outlined (12).](image-url)
to 50 μl with the electrophoretic sample buffer. For the cytoplasmic and nucleoplasmic protein fractions, the sample buffer contained 1% Triton X-100 instead of 2% Nonidet P-40. After electrophoresis the gels were fixed, impregnated with PPO, and dried under vacuum. Autoradiography was performed in time series in order to assure the comparability of the different gels.

RESULTS

Chromosomal Composition of Hybrid Cells. The chromosomal analysis that was performed by sequential staining with Giemsa/Trypsin banding and the fluorescent stain Hoechst 33258 showed the presence of a tetraploid complement of mouse erythroleukemia chromosomes and an intact human chromosome 16 in the absence of all other human chromosomes in the hybrid clone 323-4 clone 8:20 (Table 1 and Fig. 1). Electrophoresis of cell extracts and autoradiography after incubation with radioactive substrates for the enzyme APRT showed that hybrid cell 323-4 clone 8:20 contained human APRT, whereas the parent MEL cell had no APRT activity before fusion (Fig. 2). These two sets of data unequivocally establish the presence of human chromosome 16 as the only human chromosome in the hybrid cell 323-4 clone 8:20.

Analysis of Chromatin and Chromosomal Proteins. In order to avoid contamination of the chromosomal proteins with cytoplasmic or nucleoplasmic proteins, chromatin was washed and purified on a sucrose gradient. Under the conditions we used, 30% of the [35S]methionine was incorporated into cells, and a total of 20% of the initial radioactivity was finally recovered in all protein fractions. Of the labeled proteins isolated, 75% from these cell lines was of cytoplasmic origin and 10–15% was of chromosomal origin. Comparison of the two-dimensional gel patterns of proteins in the cytoplasmic, nucleoplasmic, and chromatin fractions (Figs. 3 A and B and 4 A and B) reproducibly showed in five experiments different electrophoretic patterns for each of these compartments. Most of the chromosomal proteins were found in neither cytoplasm nor nucleoplasm, and most cytoplasmic proteins were not found among chromosomal proteins, which establishes that the chromatin isolated in the manner we have described is not extensively contaminated by proteins from cytoplasmic and nuclear compartments.

Electrophoretic Separation of the Nonhistone Chromosomal Proteins in Hybrid Cells. The two-dimensional electrophoretic

![Fig. 2. Acrylamide (7.5%) electrophoresis of cell extracts after incubation with radiolabeled substrates for the enzyme APRT. Lanes: 1, mouse APRT as marker; 2, cell line 179 (tetraploid mouse erythroleukemia cell before fusion); 3, no protein added; 4, cell line 2292 (Epstein–Barr virus-transformed human lymphoblast line); 5, hybrid 323-4 clone 8:20. Cell lysates were electrophoresed in 7.5% (wt/vol) acrylamide gels, treated with radiolabeled substrates for APRT, and autoradiographed; the species origin of the enzyme was identified as described (12).](image)

![Fig. 3. Cytoplasmic (A) and nucleoplasmic (B) proteins of hybrid cell 323-4 clone 8:20. The electrophoretic pattern of these [35S]methionine-labeled proteins is different from the chromosomal proteins shown in Fig. 4.](image)

system used separates proteins having pIs between pH 4.5 and pH 7.5. Histones, which contribute about 50% of the chromosomal protein mass, were not displayed in this system because their high content of basic amino acids prevents them from entering this gel. The nonequilibrium pH electrophoresis separated them from other basic nonhistonal proteins. Both systems separated proteins in the M₆ range 10,000–150,000 as determined by marker proteins. As the denaturing agents destroy quaternary protein structures, the subunit polypeptides of most cellular proteins will be displayed by these systems. The two-dimensional gel pattern of the nonhistone chromosomal proteins from the human lymphoblast line 2292 and mouse erythroleukemia cell line 179 used to derive hybrid cell 323-4 clone 8:20 contained 280 and 400 polypeptides, respectively (Fig. 4 A and B). Although 25% of the nonhistone chromosomal proteins present in the human cell line were different from those found in the mouse cell line, the majority of chromosomal proteins in both the human and the mouse cell lines were identical.

A comparison of the two-dimensional gel electrophoresis of the hybrid cell line 323-4 clone 8:20, which contains human chromosome 16, with that of the parent MEL cell also showed that the two chromosomal protein patterns were similar but not identical (Fig. 4 B and C). There was one protein reproducibly present in the hybrid cell nonhistone chromosomal protein that never was found in the electrophoretogram of chromosomal proteins of the mouse erythroleukemia parent cell line used for fusion. This protein is identified by the small black arrow in Fig. 4 A–C. The pI of this protein was 6.2 and the M₆ was 65,000.
We next analyzed an artificial mixture of the chromosomal proteins of the human and mouse parental cells to try to identify if a chromosomal protein like the M, 65,000 protein found in the hybrid cell was present in human nonhistone chromosomal proteins (Fig. 4D). The protein of pI 6.2 M, 65,000 found in the hybrid cell 323-4 clone 8:20 was also in the human parent cell line and in two additional cell lines analyzed.

We analyzed five additional independently derived hybrid cell lines, all of which contained human chromosome 16 in addition to a tetraploid complement of mouse erythroleukemia chromosomes (see Table 1). This analysis showed that the nonhistone chromosomal protein of M, 65,000 and pI 6.2 found in hybrid 323-4 clone 8:20 was also present in four of the five additional cell lines (as shown in Table 1). The one hybrid cell that did not display this protein, JF 37-4, contained human chromosome 16 from a fibrosarcoma cell line, HT 1080. (As shown in Table 1, the human cell line contained the pI 6.2 protein before fusion.) Using selection from mass populations in increasing concentrations of dianinopurine (12), we also derived from the hybrid cell 323-4 clone 8:20 a hybrid cell line which no longer contained an intact human chromosome 16 and which was devoid of detectable APRT activity (see Table 1). This hybrid cell line is designated 323-4 clone 8:20 B-2. Two-dimensional electrophoresis of this "back-selected" hybrid showed that it also contained the M, 65,000 nonhistone chromosomal protein of pI 6.2 but in variable amounts from run to run, all of which were smaller than seen in the hybrid cell before loss of an intact human chromosome 16.

**DISCUSSION**

The data presented in this report has shown that a distinct nonhistone chromosomal protein of M, 65,000 and pI 6.2, which comigrates with a nonhistone chromosomal protein found in human cells and is absent in the mouse cells tested, was found in five out of six human–mouse somatic cell hybrids, all of which contained a tetraploid complement of mouse chromosomes in addition to human chromosome 16. The identification of this protein in five out of six independently derived hybrid cells, all of which contain human chromosome 16 and most of which contain this chromosome as the only human chromosome, strongly suggests that an association exists between the presence of hu-
man chromosome 16 and the expression of this nonhistone chromosomal protein. Although our data suggest that an association exists between human chromosome 16 and the nonhistone chromosomal protein of M, 65,000 and pl 6.2, we have not excluded the possibility that the structural gene for this protein may ultimately be found to reside on other human chromosomes as well.

The presence of this nonhistone chromosomal protein in five independently derived hybrid cells makes very unlikely the possibility that a random event, such as retention of a subchromosomal fragment of human origin by a hybrid cell, is responsible for the presence of this protein. We also had isolated hybrid clone 323-4 clone 8:20 B-2 by growth in diaminopurine which contained no APRT and did not contain an identifiable intact human chromosome 16. Although this hybrid clone exhibited far lower levels of the M, 65,000 nonhistone chromosomal protein than was present in the original hybrid cell, faint traces of this protein were clearly visible. These results may indicate (i) that a fragment of human chromosome 16 had been retained in these back-selected hybrid cells or (ii) that the gene for this protein is on more than one human chromosome, and a fragment from one of these was present in the hybrid cell and in human chromosome 16 before selection in diaminopurine.

Alternatively, this M, 65,000 nonhistone chromosomal protein could have arisen in all the hybrid cell lines from some nonspecific effect of the fusion process, which leads to the activation of expression of a mouse nonhistone chromosomal protein not present in the mouse erythroleukemia cell line used for fusion. Although this possibility is not formally excluded by our data, it is made unlikely by the absence of the M, 65,000 nonhistone chromosomal protein in the hybrid cell JF-37. A grossly intact human chromosome 16 was present in hybrid cell JF-37, and the human parent cell line HU1080, which was used to generate the hybrid cell, contained the pl 6.2 pattern. We cannot be sure of why the pl 6.2 protein was absent from this hybrid cell, although an undetected rearrangement of human chromosome 16 could have played a role.

Previous studies in interspecific somatic cell hybrids have shown both extinction and expression of genes coding for chromosomal proteins (5, 6, 14). Although some of the authors find identical nonhistone chromosomal proteins in cells of different species (6, 15), considerable differences also have been observed by others as well as ourselves (16). In our study one-fourth of the nonhistone chromosomal proteins of mouse and human cells was found to be dissimilar in the two-dimensional gel system. Thus, the results we have reported suggest that the combined use of hybrid cells containing single human chromosomes and two-dimensional gel electrophoresis may be a useful one for the study of the genetic and biological role of nonhistone chromosomal proteins. The suggestion from our data that at least some members of this protein class in interspecific somatic cell hybrids are coexpressed indicates that the system may be useful in the study of the role that these proteins may play in the regulation of differentiated gene expression.