*Proc. Natl. Acad. Sci. USA*
Vol. 77, No. 7, pp. 4079–4083, July 1980
Cell Biology

# Uniparental propagation of mitochondrial DNA in mouse–human cell hybrids

(chromosomes/isozymes/restriction enzyme/Southern hybridization)

LAURA DE FRANCESCO*, GIUSEPPE ATTARDI*†, AND CARLO M. CROCE‡

*Division of Biology, California Institute of Technology, Pasadena, California 91125; and ‡The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

*Communicated by Hilary Koprowski, March 13, 1980*

**ABSTRACT** **The retention of the two parental mitochondrial DNAs has been investigated in a large number of mouse–human cell hybrids segregating either mouse or human chromosomes, by using a highly sensitive and specific method for detection of the DNA; the results have been correlated with the karyotype and isozyme marker pattern in the same hybrid lines. In all the hybrids examined, a consistent pattern was observed for the type of mitochondrial DNA retained: the mitochondrial DNA of the parent whose chromosomes were segregated from the nucleus was undetectable or present in marginal amounts. This was true also of hybrids containing a complete set of the segregating chromosomes in the total or a large fraction of the cell population.**

Previous investigations using hybrid cells derived from fusion of cells from mice and humans (1–3), rats and humans (3), or mice and hamsters (4) have established in these hybrids a correlation, though not very strong, between chromosome and mitochondrial DNA (mtDNA) segregation. However, in none of these studies has a detailed karyological analysis of the hybrids been performed to establish the rules that govern the retention of the two parental mtDNAs in interspecific cell hybrids. Thus, it is still not known whether the disappearance of mtDNA of one parental species in the hybrid cell depends on the loss of one chromosome or set of chromosomes of that species or on an imbalance of chromosomes of the two species or on a more complex regulatory phenomenon.

A large series of hybrids between the human cell line HT-1080 and mouse cells has been isolated (5). These hybrids tend to lose chromosomes of either species depending on the mouse parent, but many of them retain in a relatively stable form a large number of chromosomes of both species. In this paper we present the results of a parallel systematic investigation of the mtDNA composition, karyotype, and isozyme marker pattern in a fairly large number of these hybrids. By using a highly sensitive and specific method for detection of mtDNA of the two parental species, we show that, in all hybrids analyzed, the mtDNA of the species being segregated from the nucleus was undetectable or present only in minute amounts.

## METHODS

**Cell Hybrids Used.** The methods for production and selection of mouse–human hybrid cell lines that lose chromosomes of either species have been described (5–7). A complete list of all hybrids analyzed in this study and the parental cell lines of each are presented in Table 1. Hybrids in group 1 were formed between a thioguanine-resistant variant (6TG) of the human cell line HT-1080 (5) and either mouse peritoneal macrophages or cells derived directly from the solid mouse teratocarcinoma OTT-6050 and were selected in hypoxanthine/aminopterin/thymidine (8). Hybrids in group 2 were formed between the cell line HT-1080 and the contact-inhibited continuous mouse cell line THO-2, which is a ouabain-resistant, hypoxanthine phosphoribosyltransferase-deficient 3T3 derivative (9), and were selected in hypoxanthine/aminopterin/thymidine supplemented with ouabain. Hybrids in group 3 were formed between an $\alpha$-amanatin-resistant variant of the human cell line HT-1080-6TG and the mouse L cell derivative clone 1D, and were selected in hypoxanthine/aminopterin/thymidine containing 7 $\mu$g of $\alpha$-amanitin per ml (Boehringer Mannheim).

**Karyologic Analysis.** Metaphase chromosomes of hybrid cells were banded according to a modification of the trypsin/Giemsa method of Seabright (10, 11).

**Isozyme Analysis.** M>H hybrid cells were studied for the expression of isozyme markers assigned to each of the different human chromosomes by starch gel or cellulose acetate gel electrophoresis (12, 13): peptidase C (PEP-C), adenylate kinase 2 (AK-2), phosphoglucomutase 1 (PGM-1), and enolase 1 (ENO-1) on chromosome 1; acid phosphatase 1 (ACP-1) and isocitrate dehydrogenase (IDH) on chromosome 2; $\beta$-galactosidase ($\beta$-GAL) on chromosome 3; phosphoglucomutase 2 (PGM-2) on chromosome 4; hexosaminidase B (HEX-B) on chromosome 5; malic enzyme (ME), phosphoglucomutase 3 (PGM-3), glyoxalase 1 (GLO-1), and superoxide dismutase 2 (SOD-2) on chromosome 6; mitochondrial malic dehydrogenase (mMDH) and $\beta$-glucuronidase ($\beta$-GUS) on chromosome 7; glutathione reductase (GTR) on chromosome 8; adenylate kinase 1 and 3 (AK-1 and -3) and mitochondrial aconitase (mACO) on chromosome 9; glutamic oxaloacetic transaminase (GOT) on chromosome 10; lactic dehydrogenase A (LDH-A) and acid phosphatase 2 (ACP-2) on chromosome 11; lactic dehydrogenase B (LDH-B) and peptidase B (PEP-B) on chromosome 12; esterase D (EST-D) on chromosome 13; nucleoside phosphorylase (NP) on chromosome 14; mannose phosphate isomerase (MPI), pyruvate kinase 3 (PK-3), and $\alpha$ subunit of hexosaminidase A (HEX-A) on chromosome 15; adenine phosphoribosyltransferase (APRT) on chromosome 16; thymidine kinase (TK) and galactokinase (GALK) on chromosome 17; peptidase A (PEP-A) on chromosome 18; glucose phosphate isomerase (PGI) on chromosome 19; adenosine deaminase (ADA) on chromosome 20; superoxide dismutase 1 (SOD-1) on chromosome 21; arylsulfatase A (ARS-A) on chromosome 22; and glucose-6-phosphate dehydrogenase (G6PD), hypoxanthine phosphoribosyltransferase (HPRT), and phosphoglycerate kinase (PGK) on the X chromosome.

H>M hybrid cells were studied for the expression of isozyme markers assigned to the following mouse chromosomes: dipeptidase 1 (DIP-1) on chromosome 1; adenylate kinase 1 (AK-1) on chromosome 2; carbonic anhydrase (CA) on chromosome 3; enolase 1 (ENO-1) on chromosome 4; $\beta$-glucuron-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: mtDNA, mitochondrial DNA.

† To whom reprint requests should be addressed.

Table 1. Hybrid cell lines analyzed

| | |
|---|---|
| 1. Hybrids losing mouse chromosomes (H>M) | |
| HT-1080-6TG × BALB/c MPM* | 55-14-F1 |
| | 55-14-F1 Cl 29 |
| | 55-14-F7 |
| | 55-54-F4 |
| | 55-91-F2 Cl 4 |
| | 55-91-F2 Cl 15 |
| HT-1080-6TG × 129 MPM III | Cl 1-7, Cl 1-13 |
| | Cl 1-15, Cl 1-16 |
| | Cl 1-17, Cl 1-20 |
| HT-1080-6TG × OTT-6050 | 55-84-F8 |
| 2. Hybrids losing human chromosomes (Mc† > H) | |
| HT-1080 × THO-2 | 56-05-F4 Cl 6 |
| | 56-05-F4 Cl 16 |
| | 56-05-F5 Cl 7 |
| | 56-05-F5 Cl 10 |
| 3. Hybrids losing mouse chromosomes (H > Mc†) | |
| HT-1080-6TG, $\alpha$-am$^R$ × Cl 1D | 58-92-F1 Cl 4 |
| | 58-92-F2 Cl 5 |
| | 58-92-F3 Cl 10 |

* MPM, mouse peritoneal macrophages.
† Mc, mouse continuous cell line.

idase ($\beta$-GUS) on chromosome 5; triphosphate isomerase (TP-1) on chromosome 6, lactic dehydrogenase A (LDH-A) on chromosome 7; glutathione reductase (GR) on chromosome 8; mannose phosphate isomerase (MPI) on chromosome 9; tripeptidase (TRIP) on chromosome 10; galactokinase (GALK) on chromosome 11; acid phosphatase 1 (ACP-1) on chromosome 12; nucleoside phosphorylase (NP) on chromosome 14; glutamic pyruvic transaminase (GPT) on chromosome 15; glyoxalase (GLO) on chromosome 17; dipeptidase 2 (DIP-2) on chromosome 18; glutamic oxaloacetic transaminase (GOT) on chromosome 19; and glucose-6-phosphate dehydrogenase (G6PD) on the X chromosome.

**Cell Growth and Labeling Conditions.** Cells were maintained as monolayers in Dulbecco's modified Eagle's medium plus 10% fetal calf serum. To provide a marker for following the DNA during purification and estimating the quantity of mtDNA recovered, cells to be used for mtDNA isolation were labeled for 2–3 days in the presence of [$^3$H]thymidine (0.2 $\mu$Ci/ml; 1 Ci = $3.7 \times 10^{10}$ becquerels).

**Isolation of mtDNA.** A mitochondrial fraction was prepared from 10–20 plates of each hybrid cell line by differential centrifugation (14). Total mtDNA was isolated by a published procedure (15). Closed circular mtDNA was isolated as described from HeLa cells (16) or from the livers and kidneys of mice.

**Restriction Digestion, Southern Blots, and DNA·DNA Hybridization.** Approximately 100 ng of hybrid cell mtDNA was digested with the restriction enzyme *Hin*cII (BioLabs) under standard conditions with a 4- to 6-fold excess of enzyme.

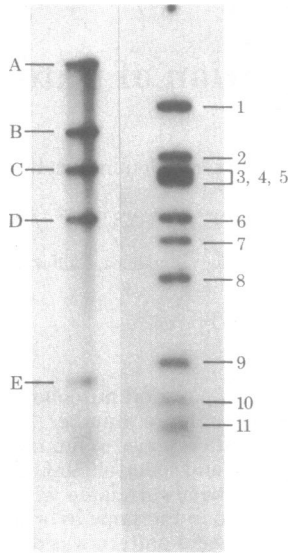


FIG. 1. Restriction enzyme patterns of human (*Right*) and mouse (*Left*) mtDNA. The autoradiograph shows the electrophoretic separation on a 1.4% agarose gel of the products obtained by *Hin*cII digestion of mtDNA from human cells and mouse liver cells. The fragments had been labeled with *Escherichia coli* DNA polymerase I and deoxynucleoside [$\alpha$-$^{32}$P]triphosphates (16).

The digest was divided into two equal portions which were run in separate lanes on a 1.4% agarose gel in Tris acetate buffer (10 mM Tris, pH 7.4/50 mM sodium acetate/2.5 mM EDTA) in parallel with *Hin*cII-digested mouse and human mtDNAs. The gels were blotted onto nitrocellulose paper by the Southern technique (17). Separate filters, each containing the hybrid mtDNA and the mouse and human mtDNA standards, were incubated with $2 \times 10^6$ cpm ($\approx$20 ng) of each parental mtDNA probe prepared essentially as described by Rigby *et al.* (18) in 4 ml of 0.9 M NaCl/0.09 M sodium citrate/0.1% $NaDodSO_4$/0.02% bovine serum albumin/0.02% Ficoll at 68°C for 20 hr in Seal-a-Meal bags (Daisey Products, Industrial Airport, K). After hybridization, the filters were washed for 10–15 min at 68°C in hybridization buffer without probe, then for 10–15 min in 75 mM NaCl/7.5 mM sodium citrate/0.1% $NaDodSO_4$ at the same temperature, and finally exposed to an x-ray film with one screen intensifier at −70°C.

## RESULTS

**Analysis of mtDNA.** The approach used in this work took advantage of both the distinctive restriction pattern of the mtDNA of each species and the base sequence differences between human and mouse mtDNAs (3, 19), increasing, therefore, the specificity of recognition of the origin of the mtDNA in the cell hybrids. Furthermore, it was possible, by this approach, to recognize directly the presence of any recombinant molecules as giving rise to fragments reacting with both parental probes. Finally, because the probes were labeled *in vitro* to a much higher specific activity (in the present work the specific activity varied between 2 and $8 \times 10^7$ cpm/$\mu$g) than obtainable in *in*

Table 2. Mouse chromosomes in H>M cell hybrids

| Hybrid | Mouse chromosome* | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1' | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | X | Markers |
| 55-14-F7 | 1.0 | 0.7 | 1.1 | 0.4 | 0.8 | 1.0 | 0.8 | 0.8 | 1.2 | 1.6 | 0.7 | 0.3 | 0.6 | 0.3 | 0.8 | 0.8 | 0.9 | 1.3 | 1.3 | 0.6 | 1.1 |
| 55-14-F1 Cl 29 | 1.0 | 0.8 | 0.6 | 0.5 | 0.9 | 0.4 | 0.9 | 1.2 | 0.5 | 0.5 | 1.1 | 0.5 | 0.1 | 0.1 | 0.1 | 0.3 | 0.8 | 0.8 | 1.0 | 0.05 | 5.2 |
| III Cl 1-7 | 0.9 | 1.2 | 0.5 | 0.4 | 1.0 | 1.8 | 0.8 | 1.6 | 1.0 | 2.0 | 1.3 | 1.2 | 1.0 | 0.9 | 0.8 | 0.4 | 1.1 | 1.2 | 1.0 | 0.5 | 3.5 |
| III Cl 1-15 | 1.2 | 0.9 | 0.9 | 0.5 | 1.2 | 1.1 | 1.1 | 1.3 | 0.8 | 1.0 | 0.6 | 0.6 | 0.5 | 0.4 | 0.2 | 0.5 | 0.3 | 0.2 | 0.3 | 0.1 | 2.0 |
| 55-84-F8 | 1.7 | 1.7 | 0.8 | 0.3 | 1.7 | 2.0 | 1.1 | 2.4 | 1.6 | 2.0 | 1.3 | 1.0 | 1.2 | 1.2 | 1.0 | 1.2 | 1.6 | 2.2 | 2.0 | 0.9 | 8.0 |
| 55-14-F1 | 0.4 | 0.4 | 1.1 | 0.7 | 0.85 | 1.1 | 0.85 | 0.7 | 0.4 | 0.8 | 0 | 0.8 | 0.6 | 0.4 | 0 | 0 | 0.3 | 0.3 | 0.6 | 0.14 | 4.6 |
| 55-54-F4 | 1.5 | 0.8 | 1.5 | 1.5 | 0.8 | 0.9 | 0.8 | 1.3 | 0.9 | 0.6 | 0.4 | 1.0 | 0.2 | 0.4 | 0.3 | 0.2 | 0.4 | 0.5 | 0.6 | 0.4 | 1.2 |
| 55-91-F2 Cl 4 | 1.1 | 0.6 | 1.7 | 1.3 | 0.6 | 0.8 | 0.6 | 1.0 | 0.45 | 0.5 | 0 | 0.4 | 0 | 0.5 | 0 | 0 | 0.1 | 0.2 | 0.45 | 0.45 | 0.6 |
| 55-91-F2 Cl 15 | 0.7 | 0.5 | 0.7 | 0.4 | 1.1 | 0.4 | 0.3 | 1.0 | 0.3 | 0.2 | 0.2 | 0.05 | 0.1 | 0.2 | 0.1 | 0.4 | 0.4 | 0.7 | 1.6 | 0.4 | 6.0 |

* Values given are the fraction of each mouse chromosome present per cell.

Table 3. Expression of mouse isozymes in H>M cell hybrids

| Hybrid | Isozymes | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DIP-1 (1) | AK-1 (2) | CA (3) | ENO-1 (4) | β-GUS (5) | TP-1 (6) | LDH-A (7) | GR (8) | MPI (9) | TRIP (10) | GALK (11) | ACP-1 (12) | NP (14) | GPT (15) | GLO (17) | DIP-2 (18) | GOT (19) | G6PD (X) |
| 55-14-F7 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 55-14-F1 Cl 29 | + | + | + | + | + | + | + | + | + | + | + | + | Weak | Weak | + | + | + | Weak |
| III Cl 1-7 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| III Cl 1-15 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | Weak |
| 55-84-F8 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 55-14-F1 | + | + | + | + | + | + | + | + | + | + | − | + | + | − | + | + | + | Weak |
| 55-54-F4 | + | + | + | + | + | + | + | + | + | + | + | + | + | Weak | + | + | + | + |
| 55-91-F2 Cl 4 | + | + | + | + | + | + | + | + | + | + | − | + | + | − | − | Weak | + | + |
| 55-91-F2 Cl 15 | + | + | + | + | + | + | + | + | + | Weak | Weak | − | + | Weak | + | + | + | + |

Abbreviations used for the isozymes are indicated in *Methods*. Numbers in parentheses are chromosome numbers.

*vivo* labeled DNA, the sensitivity of detection of minor components was greatly increased. Consequently, much smaller amounts of cells were sufficient for study than otherwise required for restriction enzyme analysis of *in vivo* labeled DNA. This advantage is particularly important to avoid the changes in the chromosome constitution of the hybrid cell population which occur during prolonged growth.

The restriction enzyme chosen for this analysis was *Hin*cII, which makes 11 cuts in human mtDNA (D. Ojala, X. Shaffer, and X. Baskir, personal communication) and 5 cuts in mouse mtDNA (19), generating fragments from both mtDNAs that are easily separated and recognized on gels (Fig. 1). Early in this work, we found that the nick-translated mtDNA probes reacted extensively with the heterologous mtDNA. However, by including a final wash in low salt at high temperature, (75 mM NaCl/7.5 mM sodium citrate; 68°C) of the blot after hybridization, it was possible in general to eliminate most of the cross-hybridization, except that involving the rRNA gene region (20). In the human pattern, the rRNA genes are located in *Hin*cII fragments 1, 6, and 7 (D. Ojala, J. Shaffer, and B. Baskir, personal communication) and, in the mouse pattern, predominantly in fragments B and D (19).

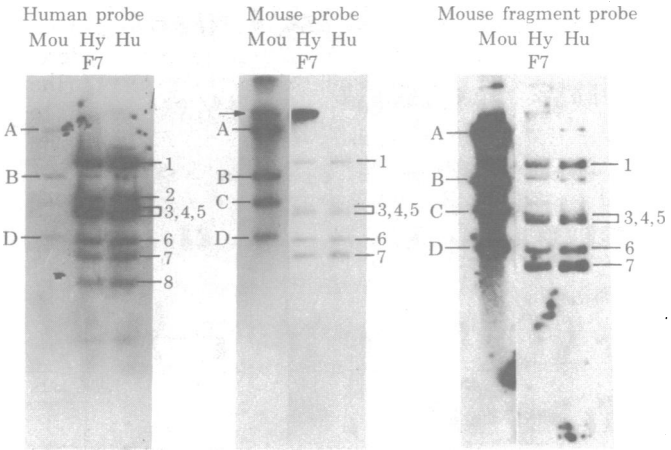


FIG. 2. Restriction fragment analysis of mtDNA from a hybrid between human HT-1080-6TG cells and BALB/c mouse macrophages, segregating mouse chromosomes (H>M). Samples of *Hin*cII-digested mtDNA from the hybrid 55-14-F7 (Hy) were run in parallel with equivalent amounts of *Hin*cII-equivalent mouse (Mou) and human (Hu) mtDNA, blotted onto nitrocellulose paper, and hybridized with $2 \times 10^6$ cpm (≈20 ng) of nick-translated human mtDNA (*Left*), mouse mtDNA (*Center*), and mouse mtDNA *Hin*cII fragments A–D (*Right*).

**mtDNA in Cell Hybrids Losing Mouse Chromosomes (H>M).** The human cell line HT-1080-6TG fused to mouse peritoneal macrophages produced hybrid cells that tended to lose mouse chromosomes and that retained the entire complement of human chromosomes (5). Several hybrids of this type (Table 1, group 1), were analyzed for the nuclear chromosome complement and for the species of mtDNA retained. Both karyotypic (Table 2) and isozyme (Table 3) analyses were done, and by both criteria, it can be estimated that a large number of mouse chromosomes were present in the majority of the cells. Representative examples of the blots of the mtDNA of these hybrids are shown in Figs. 2 and 3. The mtDNA from hybrid 55-14-F7, which was derived from the fusion of HT-1080 cells to macrophages from BALB/c mice, showed a normal human pattern when probed with human mtDNA (Fig. 2 *Left*). In this blot, it is possible to see some faint bands in the mouse standard lane, which correspond to mouse mtDNA fragments cross-hybridizing slightly with the human probe. When the hybrid mtDNA was probed with mouse mtDNA, several faint bands were observed that comigrated with bands seen in the human standard and, therefore, presumably represent cross-hybridizing human mtDNA fragments (Fig. 2 *Center*). In addition, there was a strong band that corresponded roughly to the position of uncut mtDNA (arrow in Fig. 2 *Center*). In order to investigate the identity of this band, we prepared nick-translated probes of mouse standard mtDNA from either the material corresponding to the slowly migrating band or from the combined four *Hin*cII mouse mtDNA fragments, A–D. The results of the hybridization of the latter probe with the mtDNA

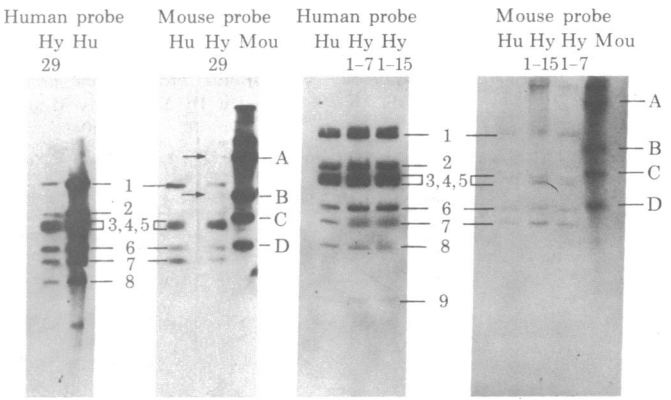


FIG. 3. Restriction fragment analysis of mtDNA from hybrids (55-14-F1 Cl 29, III Cl 1-7, and III Cl 1-15) (Hy) between human HT-1080-6TG cells and BALB/c or 129 mouse macrophages, segregating mouse chromosomes (H>M).

Table 4. Expression of human isozymes in Mc>H cell hybrids

| Human chromosome | Human isozyme marker | Mc>H hybrids (THO-2 × HT-1080) 56-05-F4 Cl 6 | 56-05-F5 Cl 10 | 56-05-F5 Cl 7 | 56-05-F4 Cl 16 |
|---|---|---|---|---|---|
| 1 | PEP-C, AK-2, PGM-1, ENO-1 | − | − | + | + |
| 2 | ACP-1, IDH | − | − | + | + |
| 3 | β-GAL | + | + | + | + |
| 4 | PGM-2 | + | + | + | + |
| 5 | HEX-B | − | − | Weak + | − |
| 6 | ME, PGM-3, GLO-1, SOD-2 | − | − | + | + |
| 7 | mMDH, β-GUS | + | + | + | − |
| 8 | GTR | + | + | + | − |
| 9 | AK-1, AK-3, mACO | + | + | − | + |
| 10 | GOT | + | + | + | + |
| 11 | LDH-A, ACP-2 | + | + | + | + |
| 12 | LDH-B, PEP-B | + | + | + | + |
| 13 | EST-D | − | + | Weak + | + |
| 14 | NP | + | + | + | + |
| 15 | MPI, PK-3, HEX-A | + | + | Weak + | + |
| 16 | APRT | + | + | + | + |
| 17 | TK, GALK | + | + | Weak + | + |
| 18 | PEP-A | + | − | − | + |
| 19 | PGI | + | + | + | + |
| 20 | ADA | + | + | + | − |
| 21 | SOD-1 | + | + | + | + |
| 22 | ARS-A | + | − | − | − |
| X | G6PD, HPRT, PGK | + | Weak + | + | + |

Abbreviations used for the isozymes are indicated in *Methods*.

of the hybrid and with the standard DNAs are shown in Fig. 2 *Right*. With this probe, hybridization to the slowly migrating component has been nearly completely eliminated, indicating that this component probably does not contain mouse mtDNA sequences. In support of this conclusion, when the probe prepared from the slowly moving component was annealed with *Hin*cII-digested mouse mtDNA, no hybridization was observed to mouse fragments A–E, but a strong band of hybridization was observed at the position of the large component (not shown). The band with the mobility of mouse mtDNA fragment B appearing in the restriction pattern of the F7 mtDNA hybridized with human probe (Fig. 2 *Left*) or mouse probe (Fig. 2 *Right*) probably represents a human mtDNA partial, because it is present also in the human standard and because no bands corresponding to the other mouse mtDNA fragments are observed (the same partial is visible in the blots in Fig. 6).

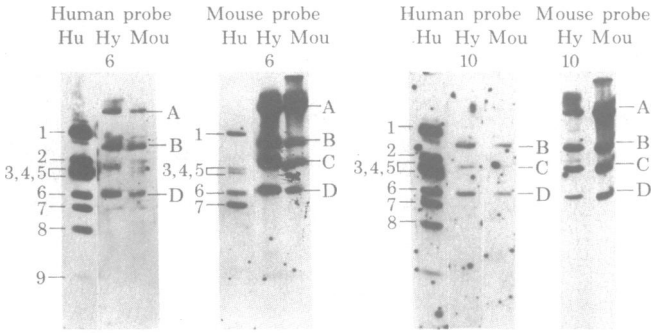


FIG. 4. Restriction fragment analysis of mtDNA from hybrids (56-05-F4 Cl 6 and 56-05-F5 Cl 10) (Hy) between human HT-1080 cells and the continuous mouse cell line THO-2, segregating human chromosomes (Mc>H).

In Fig. 3 *Left*, hybrid 55-14-F1 Cl 29, which has the same parents as the previous hybrid but lacks a larger number of mouse chromosomes (Tables 2 and 3), is shown to have a normal human mtDNA pattern when probed with human mtDNA. In the blot incubated with mouse mtDNA probe, it is possible to see very faint mouse mtDNA bands (arrows), which probably represent less than 1% of the total mtDNA in the hybrid, in addition to the more prominent cross-hybridizing human mtDNA fragments. In Fig. 3 *Right* are shown two other hybrids formed by the fusion of HT-1080-6TG and mouse macrophages from strain 129. Even upon very long exposure of the autoradiogram, there were no mouse mtDNA fragments visible.

When HT-1080-6TG cells were fused with mouse teratocarcinoma cells (OTT-6050), hybrid cells losing mouse chromosomes were also obtained (6). A single hybrid of this type was analyzed (55-84-F8) (the chromosome and isozyme patterns are shown in Tables 2 and 3, respectively). Here again, a normal human mtDNA pattern was seen with the human mtDNA probe, and no mouse mtDNA fragments were detected in the hybridization with the mouse mtDNA probe.

**mtDNA in Hybrids Losing Human Chromosomes (Mc>H).** When human HT-1080 cells were fused with the continuous mouse cell line THO-2, hybrids were obtained that preferentially lost human chromosomes upon continuous culture, but many of the hybrids retained large numbers of human chromosomes (5). In the hybrids investigated here (Table 1), from the analysis of the isozymes present it would be inferred that between 16 and 19 of the human chromosomes were present in the majority of the cells (Table 4). As shown in Fig. 4 *Left*, hybrid 56-05-F4 Cl 6 revealed a normal mouse mtDNA pattern; even upon long exposure of the autoradiogram, the blot hybridized with human mtDNA showed no human mtDNA fragments. The faint band with the mobility of human fragment 7 is presumably a contaminant because it is also present in the mouse standard. Hybrid 56-05-F5 Cl 10 gave a similar result (Fig. 4 *Right*). The faint bands visible between the A and B and between the B and C mouse fragments are not human

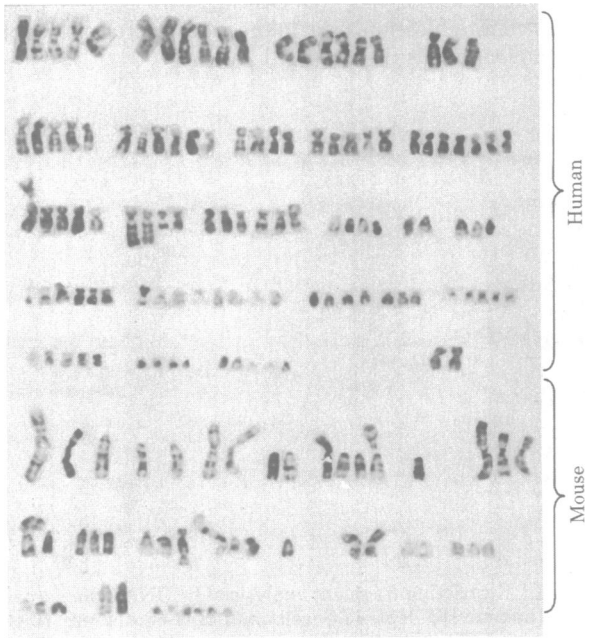


FIG. 5. Karyotype of a hybrid clone between α-amanitin-resistant HT-1080-6TG human fibrosarcoma cells and Cl 1D mouse cells. Almost all of the mouse Cl 1D chromosomes (Cl 1D cells have approximately 50 mouse chromosomes) are present in the hybrid. Six small marker chromosomes of unknown origin (shown at the end of the mouse set) are present in the hybrids.

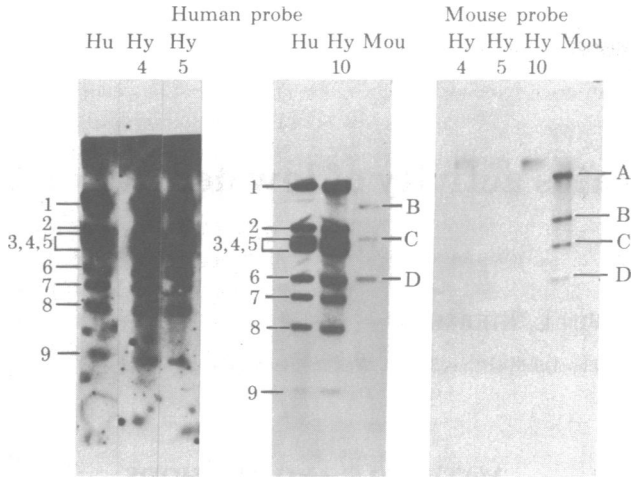


FIG. 6. Restriction fragment analysis of mtDNA from hybrids (58-92-F1 Cl 4, 58-92-F2 Cl 5, and 58-92-F3 Cl 10) (Hy) between α-amanitin-resistant human HT-1080-6TG cells and the continuous mouse cell line clone 1D, segregating mouse chromosomes (H>Mc).

mtDNA fragments, but rather either contaminants or mouse mtDNA partials; they are not visible after hybridization with the human probe and no other human fragments are visible.

**mtDNA in Hybrids Formed Between α-Amanitin-Resistant Human Cells and Mouse LM (TK⁻) Cl 1D Cells (H>Mc).** In all hybrids derived from the fusion of established lines of mouse and human cells, the human chromosomal complement is unstable and human chromosomes tend to be shed during continued culturing. However, when α-amanitin-resistant human HT-1080-6TG cells were fused with mouse clone 1D cells and hybrids selected in the presence of α-amanitin, the entire chromosomal complement of human origin was retained in an apparent stable state and the mouse chromosomes showed only a very slow loss; these hybrids expressed only human 28S rRNA (M. Shander and C. Croce, unpublished data). Several hybrids of this kind (Fig. 5), which had a complete human and mouse chromosome complement in the majority of the cells, were analyzed for the species of mtDNA retained; only human mtDNA was detected (Fig. 6).

## DISCUSSION

In all cell hybrids analyzed in the present work, regardless of the chromosome composition and direction of chromosome loss, a single species of mtDNA was detected, and this belonged to the parent whose nuclear chromosomes were more stable. In one set of hybrids (formed between α-amanitin-resistant human cells and α-amanitin-sensitive mouse cells), there was at least one copy of each mouse chromosome, in addition to several copies of each human chromosome, in most hybrid cells. This result makes it unlikely that the absence of a specific chromosome or set of chromosomes of one parent is responsible for the inability of the hybrid cells to replicate and maintain the mtDNA of that parent and is suggestive of some kind of regulation of gene expression in the hybrid cells.

Selective suppression of gene expression at other loci has been previously found in mouse–human hybrid cells. Thus, the nuclear rRNA genes of a single species (7, 21) are transcribed in spite of the presence of both sets of rRNA genes in the hybrid cells, as shown by genomic blots (22). In addition, the functions required for the replication of species-specific viruses that are controlled by the unstable parent are suppressed (23). It is clear, however, that suppression of these functions does not entail the inactivation of the entire genome, because marker isozymes of the suppressed parent, which are coded for by genes distributed on nearly all chromosomes, can be detected in hybrid cells. The nature of the suppression at the molecular level is unknown.

The results obtained with the cell hybrids analyzed here agree with previous studies in which the retention of a single species of mtDNA was demonstrated (1, 2). However, in the studies on rodent–human hybrid cells performed by Coon *et al.* (3), several cell hybrids were found in which both species of parental mtDNA were detected. The reason for this discrepancy is not clear. It seems likely that the mouse–human cell hybrids are in all cases unable to replicate both species of mtDNA indefinitely and that the hybrids analyzed in the cited study had not yet reached the point of complete elimination of one species of mtDNA. It would be important to establish whether hybrid cells are indeed capable of propagating mtDNA even for a limited time, as this would suggest a different mechanism of suppression of the genes required for mtDNA retention from that operating on the rRNA genes; the inactivation of rRNA genes has, in fact, been shown to occur within very few cell generations after nuclear fusion (24).

This work was supported by Research Grant PCM-7718037 from the National Science Foundation and a Junior Fellowship of the American Cancer Society, California Division (to L.D.), and by grants from the National Institutes of Health and the National Foundation–March of Dimes.

1. Clayton, D. A., Teplitz, R. L., Nabholz, U., Dovey, H. & Bodmer, W. (1971) *Nature (London)* **234**, 560–562.
2. Attardi, B. & Attardi, G. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2874–2878.
3. Coon, H. G., Horak, I. & Dawid, I. B. (1973) *J. Mol. Biol.* **81**, 285–298.
4. Eliceiri, G. L. (1973) *Nature (London) New Biol.* **241**, 233–234.
5. Croce, C. M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3248–3252.
6. Miller, O. J., Miller, D. A., Dev, V. G., Trantravahi, R. & Croce, C. M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4531–4535.
7. Croce, C. M., Talavera, A., Basilico, C. & Miller, O. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 694–697.
8. Littlefield, J. W. (1964) *Science* **145**, 709–710.
9. Jha, K. K. & Ozer, H. L. (1976) *Somatic Cell Genet.* **2**, 215–223.
10. Seabright, M. (1971) *Lancet* ii, 971–972.
11. Croce, C. M., Kieba, I. & Koprowski, H. (1973) *Exp. Cell Res.* **79**, 461–463.
12. Chern, C. & Croce, C. M. (1976) *Am. J. Hum. Genet.* **28**, 350–356.
13. O'Brien, D., Linnenbach, A. & Croce, C. M. (1978) *Cytogenet. Cell Genet.* **21**, 72–76.
14. Attardi, B., Cravioto, B. & Attardi, G. (1969) *J. Mol. Biol.* **43**, 47–117.
15. Storrie, B. & Attardi, G. (1972) *J. Mol. Biol.* **71**, 177–199.
16. Ojala, D. & Attardi, G. (1977) *Plasmid* **1**, 78–105.
17. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
18. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
19. Parker, R. C. & Watson, R. M. (1977) *Nucleic Acids Res.* **4**, 1291–1300.
20. DeFrancesco, L. & Attardi, G. (1980) *J. Mol. Biol.*, in press.
21. Eliceiri, G. L. & Green, H. (1969) *J. Mol. Biol.* **41**, 253–260.
22. Perry, R. P., Kelley, D. E., Schibler, U., Huebner, K. & Croce, C. M. (1979) *J. Cell. Physiol.* **98**, 553–560.
23. Huebner, K., Shander, M. & Croce, C. M. (1977) *Cell* **11**, 25–33.
24. Marshall, C. J., Handmaker, S. D. & Bramwell, M. E. (1975) *J. Cell Sci.* **17**, 307–325.