

Loss of mouse chromosomes in somatic cell hybrids between HT-1080 human fibrosarcoma cells and mouse peritoneal macrophages

(chromosome segregation/isozymes/transformed fibroblasts)

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ABSTRACT Somatic cell hybrids between mouse peritoneal macrophages and HT-1080 human fibrosarcoma cells lose mouse chromosomes and retain the entire complement of human chromosomes. In contrast, somatic cell hybrids between cells derived from two different mouse continuous cell lines and HT-1080 human cells were found to lose human chromosomes preferentially. Loss of mouse chromosomes is not a general property of hybrids between mouse macrophages and transformed human cells; the hybridization of mouse macrophages with cells derived from five different human fibroblast lines transformed by simian virus 40 resulted in the production of hybrid clones that preferentially lost human chromosomes.

When mouse cells from established cell lines are fused with human cells, hybrid cells are formed that preferentially lose human chromosomes (1). The loss of human chromosomes from the hybrid cells appears to be nonrandom (2-4). Fusion of mouse peritoneal macrophages (MPM) with simian virus 40 (SV40)-transformed human cells also produces hybrid cells that lose human chromosomes and retain the entire complement of mouse chromosomes (5, 6); such hybrids selectively retain the human chromosome carrying the SV40 genome (7), while they lose the other human chromosomes (5, 6). Since all these hybrids are transformed *in vitro* (5, 6) and are tumorigenic when injected into *nude* mice (8), it would appear that the human chromosome carrying the genome of SV40 is responsible for the expression of the transformed phenotype *in vitro* and of the malignant phenotype *in vivo* (5, 6). More recently, however, Minna and Coon reported that the fusion of SV40-transformed human cells with mouse embryonic cells results in hybrid cells that preferentially lose mouse chromosomes, while retaining the entire complement of human chromosomes (9).

Cell lines derived from a number of different human cancers have been established in tissue culture (10). One of these lines, HT-1080, derived from a 35-year-old Caucasian male, is particularly interesting, since the majority of its cells contain 46 chromosomes and very few chromosomal rearrangements (10). HT-1080 human fibrosarcoma cells form colonies in semisolid media (10) and induce tumors in immunosuppressed animals (11).

Since the human chromosome carrying the SV40 genome is selectively retained in hybrids between mouse macrophages and SV40-transformed human cells (5, 6), it was of interest to determine whether somatic cell hybrids between MPM and HT-1080 human fibrosarcoma cells would also retain a specific human chromosome, and whether these hybrid cells would be transformed *in vitro* and be tumorigenic when injected into immunosuppressed mice. Such a human chromosome would

be responsible for the transformed and malignant phenotype of these human fibrosarcoma cells.

MATERIALS AND METHODS

Cells. HT-1080 human fibrosarcoma cells, obtained from A. J. Girardi, were selected in Eagle's minimal essential medium containing 30 $\mu\text{g}/\text{ml}$ of 6-thioguanine. A drug-resistant line, HT-1080-6TG, so isolated, was deficient in hypoxanthine phosphoribosyltransferase (HPRT) (EC 2.4.2.8) and died in hypoxanthine-aminopterin-thymidine (HAT) medium (12).

THO-2 cells are contact-inhibited BALB/c mouse fibroblasts deficient in HPRT and resistant to ouabain (13). IT-22 cells are contact-inhibited mouse fibroblasts deficient in thymidine kinase (TK) (EC 2.7.1.75) (2). OTT6050 cells were derived from a solid teratocarcinoma in a 129 mouse (14, 15), obtained from B. Mintz, Institute for Cancer Research, Fox Chase, Pa. MPM were obtained from BALB/c, C57BL/6, and 129 mice by a modification of the method described by Cohn and Benson (16). LN-SV and SV-Victor cells are Lesch-Nyhan fibroblasts transformed by SV40 (17). GM54VA and GM52VA cells, obtained from the Institute for Medical Research, Camden, N.J., and HPRT-W18Va2 cells are human fibroblasts transformed by SV40.

Fusion and Selection of Hybrids. Human and mouse cells were fused in the presence of β -propiolactone-inactivated Sendai virus at pH 8.0 (18). HT-1080 fibrosarcoma cells were fused with THO-2 cells and the hybrids were selected in HAT medium containing 0.1 mM ouabain. Resistance to ouabain is a dominant trait (19). Hybrids between HT-1080-6TG cells and either IT-22 cells or MPM were selected in HAT medium (MPM are nondividing cells). HT-1080-6TG cells were also fused with OTT6050 cells and the hybrids were selected in HAT medium.

The procedures used to obtain somatic cell hybrids between MPM and HPRT⁻ SV40-transformed human cells have been described elsewhere (5, 6). Hybrids between either GM54VA or GM52VA cells and IT-22 cells and MPM were selected in HAT medium containing 0.1 mM ouabain, and in minimal essential medium containing 0.1 mM ouabain, respectively.

Fusion of HT-1080-6TG cells with either MPM or OTT6050 cells resulted in the appearance of hybrid cell colonies in HAT selective medium after 3-4 weeks. Each hybrid clone was derived from a different colony. The hybrid clones between HT-1080-6TG and BALB/c MPM were derived from four different fusion experiments.

Karyologic Analysis. Human and mouse chromosomes were identified in the hybrid clones by a modification of the trypsin-Giemsa banding technique described by Seabright (20). At least 15 metaphases were photographed and analyzed per hy-

Abbreviations: MPM, mouse peritoneal macrophages; SV40, simian virus 40; HPRT, hypoxanthine phosphoribosyltransferase; HAT, hypoxanthine-aminopterin-thymidine.

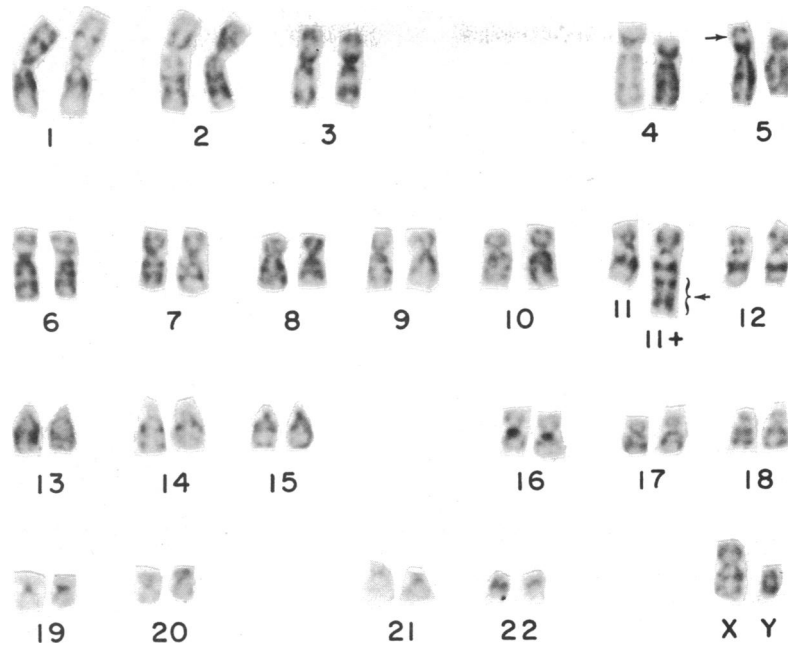


FIG. 1. Karyotype of an HT-1080-6TG cell. Most of the human chromosomes appear to be normal with the exception of one chromosome 5 that has additional material on the short arm and one chromosome 11 that has additional material on the long arm.

brid clone. The results of the karyologic analysis of most of the LN-SV \times MPM and W18Va2 \times MPM hybrids have been reported (6).

Isozyme Analysis. Hybrid clones were analyzed for the following isozymes: mannosephosphate isomerase (MPI) (EC

5.3.1.8), which has been assigned to chromosome 15 in man (21); β -glucuronidase (GUS) (EC 3.2.1.31), assigned to chromosome 5 in the mouse (22) and to chromosome 7 in man (23); nucleoside phosphorylase (NP) (EC 2.4.2.1), assigned to chromosome 14 in man (24); and glucosephosphate isomerase (GPI)

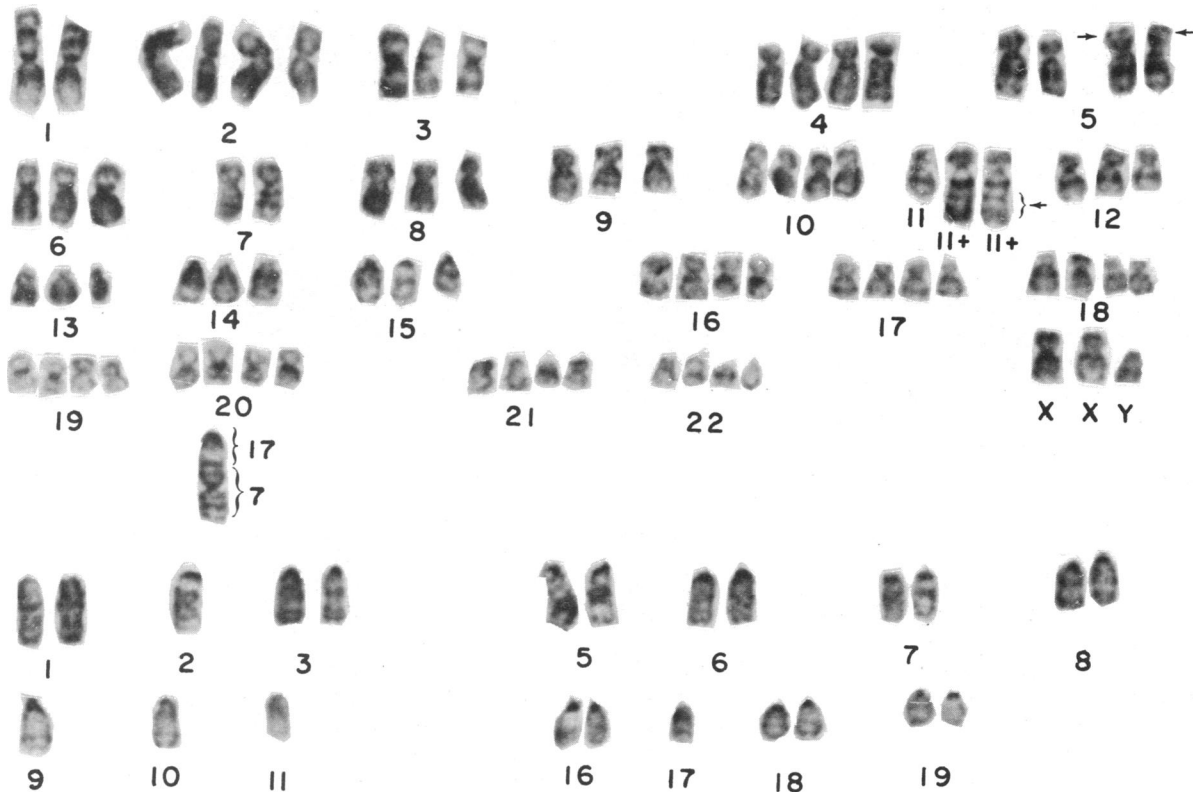


FIG. 2. Karyotype of a BALB/c MPM \times HT-1080-6TG hybrid originally selected in HAT medium and subsequently carried in minimal essential medium for 45 passages. Mouse chromosomes 4, 12, 13, 14, 15, X, and Y are absent from this hybrid cell. Three to four copies of each human autosome with the exception of chromosome 1 are present in the hybrid. A chromosome marker formed by the fusion of mouse chromosome 17 and human chromosome 7 is also present. The arrows indicate the two human chromosome markers.

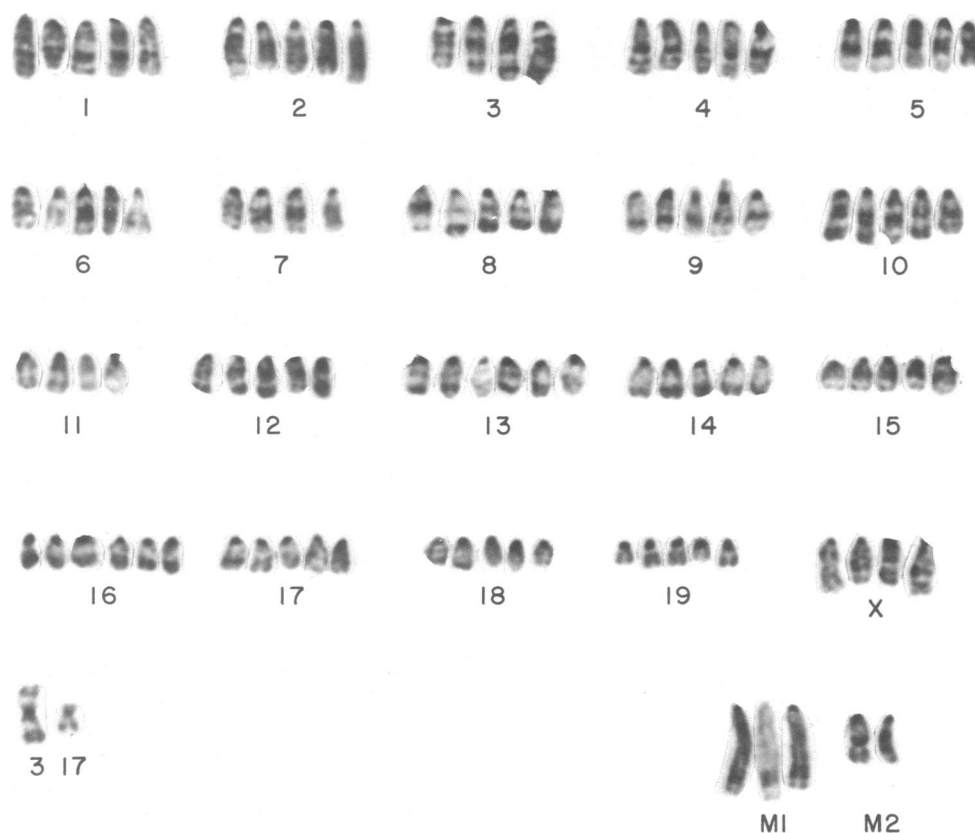


FIG. 3. Karyotype of an IT-22 \times HT-1080-6TG hybrid. Only two human chromosomes are present in this hybrid (chromosomes 3 and 17) (last row). All other chromosomes are of mouse origin. M1 and M2 are two chromosome markers of the IT-22 cells.

(EC 5.3.1.9), assigned to chromosome 7 in the mouse (25) and to chromosome 19 in man (26). The starch gel and Cellophane electrophoresis procedures used in this study have been described elsewhere (23–26).

RESULTS

Karyologic analysis of the hybrid cells

The HT-1080-6TG cell line is heteroploid with a modal number of 46 chromosomes (Fig. 1). Two chromosome rearrangements can be detected in this human cell line: one involving chromosome 5 and the other involving chromosome 11 (indicated by the arrows). Karyologic analysis of the parental HT-1080 cell line gave identical findings (10). All 18 hybrids between HT-1080-6TG cells and MPM retained the entire complement of human chromosomes and lost mouse chromosomes (Fig. 2; Table 1). In general, more than two copies of each human chromosome were observed in the hybrid clones (Fig. 2). Mouse chromosomes were eliminated from the hybrid cells but at least a copy of most of the mouse chromosomes was retained by the majority of the hybrid clones. The karyotype of a hybrid clone that has lost mouse chromosomes 4, 12, 13, 14, 15, X, and Y is shown in Fig. 2. Interestingly, mouse chromosomes 12 and 14 appeared to be absent from six of the 18 hybrid clones. Mouse chromosomal loss was also observed in two clones derived from the fusion of HT-1080-6TG cells and cells directly derived from a solid teratocarcinoma (OTT6050) (15). In one of these clones, 55–84 F8 Cl 3, mouse chromosome 12 was missing from approximately 80% of the hybrid cells. In the other clone, 55–84 F8 Cl 8, mouse chromosome 12 and 14 were completely missing. In contrast, all 16 somatic cell hybrids between HT-1080 or HT-1080-6TG cells and contact-inhibited IT-22 or THO-2

mouse cells preferentially lost human chromosomes, and no more than 11 different human chromosomes were retained by the hybrid clones (Fig. 3; Table 1). These experiments indicate that the loss of mouse chromosomes is a characteristic of hybrids of HT-1080 human fibrosarcoma cells with MPM, and with primary mouse teratocarcinoma cells.

Since human fibrosarcoma cells are transformed *in vitro* and

Table 1. Loss of chromosomes from mouse–human somatic cell hybrids

Parental cells		Number of clones* losing	
Human	Mouse	Human chromosomes	Mouse chromosomes
HT-1080-6TG	\times IT-22	10/10	0/10
HT-1080	\times THO-2	6/6	0/6
HT-1080-6TG	\times BALB/c MPM	0/14	14/14
HT-1080-6TG	\times C57BL MPM	0/4	4/4
HT-1080-6TG	\times OTT6050	0/2	2/2
LN-SV	\times BALB/c, C57BL and 129 MPM	73/73	0/73
W18Va2	\times BALB/c and C57BL MPM	37/37	0/37
SV-Vector	\times BALB/c and C57BL MPM	6/6	0/6
GM52VA	\times BALB/c and C57BL MPM	5/5	0/5
GM54VA	\times BALB/c and C57BL MPM	24/24	0/24
LN-SV	\times IT-22	14/14	0/14
GM54VA	\times IT-22	12/12	0/12

* Over total analyzed.

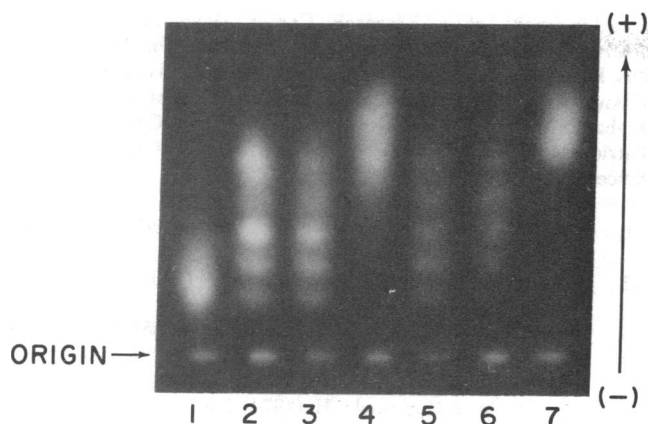


FIG. 4. Zymogram of β -glucuronidase on Cellogel (cellulose acetate). An extract of BALB/c THO-2 cells is in lane 4; HT-1080-6TG human cells are in lane 1; THO-2 \times HT-1080-6TG hybrid cells that have lost human chromosome 7 are in lane 7. Four different BALB/c MPM \times HT-1080-6TG hybrid clones (lanes 2, 3, 5, and 6) express both mouse and human β -glucuronidase and three heteropolymers between the mouse and the human enzyme.

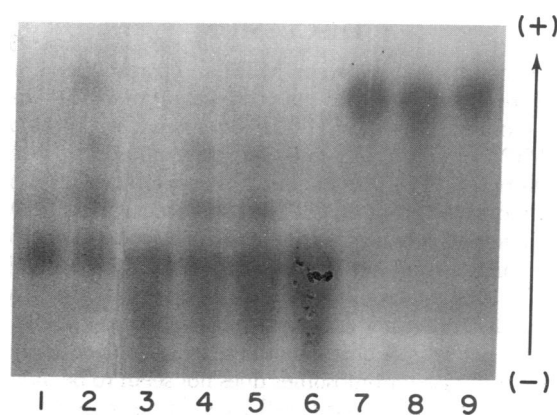


FIG. 6. Zymogram of nucleoside phosphorylase in starch gel. HT-1080-6TG is in lane 6. Three hybrid clones between THO-2 and HT-1080 cells that have lost human chromosome 14 are in lanes 7, 8, and 9. Two hybrid clones between OTT6050 and HT-1080-6TG cells are in lanes 1 and 2. The hybrid clone in lane 1 expresses the human enzyme and, very weakly, heteropolymers between the human and the mouse enzyme. The clone in lane 2 expresses both the human and the mouse enzyme and two heteropolymers. Two hybrid clones between BALB/c MPM and HT-1080-6TG cells express the human enzyme and heteropolymers between the human and the mouse enzyme (lanes 4 and 5). No mouse nucleoside phosphorylase is expressed in the BALB/c MPM \times HT-1080-6TG hybrid clone in lane 3.

tumorigenic *in vivo* it was of interest to note that hybrid clones between MPM and other transformed human cells lose human and not mouse chromosomes. This has been shown in other publications from this laboratory in somatic cell hybrids of MPM with the SV40-transformed human cells LN-SV and W18Va2, which lose only human chromosomes and retain the entire mouse chromosome complement (Table 1) (5, 6). Somatic cell hybrids between three additional SV40-transformed human cells and MPM also lost human chromosomes (Table 1) (C. M. Croce, manuscript in preparation). Somatic cell hybrids between contact-inhibited mouse fibroblasts and SV40-transformed human cells preferentially lost human chromosomes (Table 1). These experiments indicate that the loss of mouse chromosomes is not a general property of hybrids between MPM and transformed human cells.

Isozyme analysis

Hybrid cell clones of HT-1080-6TG with either MPM or OTT6050 cells were also studied for the expression of mouse constitutive isozymes to determine whether the loss of specific mouse chromosomes was paralleled by the loss of these enzymes. As shown in Fig. 4, the hybrids between HT-1080-6TG human fibrosarcoma cells and MPM retained both mouse and human forms of the enzyme β -glucuronidase and expressed three heteropolymers of the mouse and human enzymes. Twelve

independent HAT-selected hybrid clones between BALB/c MPM and HT-1080-6TG cells were found to express both the mouse and the human β -glucuronidase, confirming the results of the karyologic analysis that mouse chromosome 5 is retained by these hybrids.

As shown in Fig. 5, mouse mannose phosphate isomerase was expressed weakly in four HT-1080-6TG \times MPM hybrid clones. Mouse nucleoside phosphorylase activity and mouse-human heteropolymers of the enzyme segregated in the hybrid clones between HT-1080-6TG cells and mouse OTT6050 cells and between HT-1080-6TG cells and MPM (Fig. 6). Mouse nucleoside phosphorylase was not expressed in the clone 55-14 F7 Cl 3 (Fig. 6), which has lost mouse chromosomes 4, 12, and 14. Segregation of mouse glucosephosphate isomerase activity was also observed in the hybrid clones (Fig. 7). Loss of mouse chromosome 7 was observed in the clone 55-14 F1 Cl 1, which did not express mouse glucosephosphate isomerase activity (Fig. 7, lane 5).

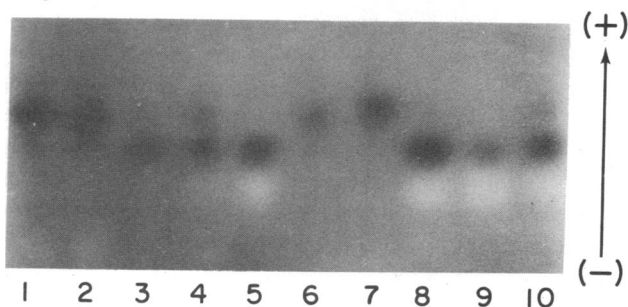


FIG. 5. Zymogram of mannosephosphate isomerase in starch gel (dark bands). Extracts of HT-1080 and HT-1080-6TG cells are in lanes 5 and 9. Four mouse-human hybrid clones between HT-1080-6TG cells (lane 1) and between BALB/c MPM and LN-SV cells (lanes 2, 6, and 7) express only the mouse form of the enzyme. Mouse mannosephosphate isomerase is weakly expressed in four BALB/c MPM \times HT-1080-6TG hybrid clones (lanes 3, 4, 8, and 10).

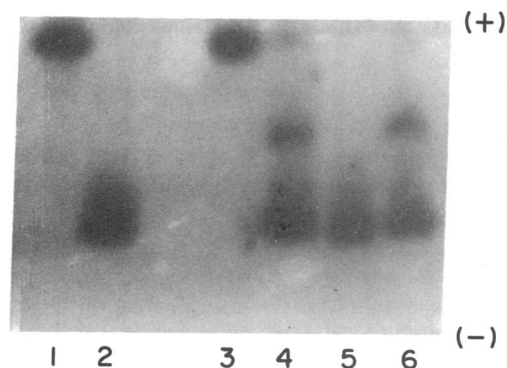


FIG. 7. Zymogram of glucosephosphate isomerase in starch gel. An extract of a BALB/c MPM \times LN-SV hybrid containing only human chromosome 7 and no other human chromosomes is in lane 1. An extract of HT-1080-6TG cells is in lane 2; OTT6050 cells, lane 3. Hybrid clones 55-14 F7 Cl 45 (lane 4) and 55-14 F7 Cl 3 (lane 6) express the human and the mouse form of the enzyme and one heteropolymer. Hybrid clone 55-14 F1 Cl 1 (lane 5) expresses only the human enzyme.

DISCUSSION

Somatic cell hybrids between MPM and transformed human cells were investigated for the presence of mouse and human chromosomes. Hybrids of HT-1080 human fibrosarcoma cells with MPM preferentially lose mouse chromosomes, as do hybrids between HT-1080 human fibrosarcoma cells with cells directly derived from an OTT6050 teratocarcinoma. On the other hand, all somatic cell hybrids between cells of two different contact-inhibited mouse cell lines (IT-22 and THO-2) and HT-1080 human fibrosarcoma cells preferentially lose human chromosomes. At present, it is not known why these different patterns of chromosome segregation occur.

Loss of mouse chromosomes does not seem to be, however, a characteristic of somatic cell hybrids between MPM and transformed human cells, since hybrids between MPM and five different SV40-transformed human cells preferentially lose human chromosomes and retain the entire complement of mouse chromosomes.

Since mouse chromosomes are preferentially lost in the HT-1080 × MPM hybrid cells, these hybrids could be used in the assignment of genes to mouse chromosomes and in studies of the genetics of mouse endogenous type-C RNA tumor viruses, because it should be possible to correlate the inducibility of ecotropic and xenotropic murine type-C RNA tumor viruses with the presence of specific mouse chromosomes in the hybrid clones.

In addition, these hybrids should be useful in studies of genetic control of malignancy. The study of the hybrids between HT-1080 human fibrosarcoma cells and MPM should indicate whether the normal mouse genome contains gene(s) capable of suppressing the malignant phenotype in fibrosarcoma cells, or whether all these hybrids are transformed *in vitro* and are tumorigenic when injected into *nude* mice (8). If dominant expression of the malignant phenotype occurs in the hybrids of this type, including those which have retained all mouse chromosomes, the study of the tumorigenicity of the hybrids between HT-1080 human fibrosarcoma cells and non-tumorigenic IT-22 and THO-2 mouse cells should result in the identification of the human chromosome(s) responsible for malignant transformation in HT-1080 human fibrosarcoma cells.

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