Somatic Cell Hybrid between the Established Human Line D98 (Presumptive HeLa) and 3T3

Abstract. *Somatic cell hybrids have been made between an established human cell line with a long culture history and an established mouse fibroblast line. When first analyzed, the hybrid cells contained nearly twice as many mouse chromosomes as the mouse parent line, and a human chromosome complement of about half that of the human parent. There was further loss of human chromosomes on continued cultivation. This behavior resembles that of other human-mouse hybrids and appears to be characteristic of the human-mouse combination. However, the number of human chromosomes is greater than in hybrids made from human diploid fibroblasts. Some clones contain more than a haploid quantity of human DNA per cell and should synthesize a much greater number of human gene products.*

Among the somatic cell hybrids produced in culture, the majority contain most or all of the chromosomes present in the original parent cells (*1*). However, hybrids produced between human diploid fibroblasts and an established line of mouse cells behave differently (*2*, *3*). They maintain the entire chromosome complement of the mouse parent cell, but during cultivation progressively lose human chromosomes. The same process occurs in human-mouse hybrids made with human fibroblasts that had previously been transformed with the oncogenic virus SV40 (*4*). Loss of human chromosomes is a very valuable property of the hybrids since it permits chromosomal assignment of human genes (*2*, *3*). However, the preferential loss of human chromosomes is puzzling, and because of it there are no human-animal cell hybrids available which could synthesize a full set of human gene products.

The human parent cells used in these crosses were all of fibroblast origin, and all had a relatively short culture history compared with the mouse parent cl 1D, a derivative of L cells, which has been in culture for about 25 years (*5*). The possibility was considered that elimination of human chromosomes might be related to a comparatively poor adaptation of the human parent cells to culture conditions. If so, a hybrid between an old and well-established human line and a relatively recently established mouse line might be expected to maintain the human chromosome complement and possibly to lose mouse chromosomes.

A thymidine kinase-less (TK$^-$) descendant of the mouse line 3T3 (*6*) was prepared by serial culture of 3T3-4 (a clone of 3T3) in the presence of bromodeoxyuridine (BUDR) as described previously for other mouse lines (*7*). Cells were first plated in the presence of BUDR (5 μg/ml), which killed most of them. Two of the colonies that grew out were thereafter serially transferred as separate lines. After four transfers in medium containing BUDR (5 μg/ml), each line was carried for 14 transfers in the presence of BUDR at a concentration of 30 μg/ml, first at a low cell dilution while the cells grew poorly and later at higher dilutions as growth improved. One clone was then isolated from each line, and they were designated 3T3-4(E) and 3T3-4(C2). These lines were able to grow with a doubling time of 20 hours; the growth rate was not affected by 30 μg of BUDR per milliliter. The cells were deficient in thymidine kinase and were killed in HAT medium (hypoxanthine, aminopterin, and thymidine) (*8*, *9*).

The karyotype of 3T3-4(E) is shown in Fig. 1A. All the chromosomes are telocentric in type, which is characteristic of the mouse; no metacentrics have been introduced by chromosomal rearrangements, as often occurs in long-established mouse lines (*10*). The chromosome number is in the subtetraploid range, as previously reported for wild type 3T3 (*6*). However, in the 3T3 TK$^-$ variants, as in TK$^-$ L cells (*7*), there was a reduction in modal chromosome number, in this case from 72 (characteristic of the wild type) to 68 or 69.

The human parent line D98/AH2 (*8*), which must be considered a descendant of HeLa (*11*), lacks hypoxanthine phosphoribosyl transferase and could be used together with 3T3 (TK$^-$) in the double selective system of Littlefield (*9*). The karyotype of D98/AH2 is shown in Fig. 1B. It contains a mode of 63 human chromosomes, most of which are typical. The majority are metacentric or submetacentric and are easily distinguishable from 3T3 chromosomes. Those of groups D and G are acrocentrics, and are difficult to distinguish from mouse chromosomes.

Two cell mixtures were prepared for hybridization, one of D98 $\times$ 3T3-4(E) and one of D98 $\times$ 3T3-4(C2), by inoculating 5 $\times$ 10^5 cells of each type together into petri dishes. Twenty-four hours later the contents of each dish were transferred at dilutions of 1:5, and HAT medium was added to each. Hybrid colonies developed at a frequency of about eight per 10^5 of 3T3-4(E) and about two per 10^5 of 3T3-4(C2). Ten clones of D98/3T3-4(E) hybrids (designated as HLE) were isolated, and the nature of their chromosome complement was examined in metaphase preparations.

Figure 2 is a frequency distribution diagram of the number of human type and mouse type chromosomes in parents and hybrids. Unexpectedly, all hybrids contained more than a full complement of 3T3 type chromosomes—usually close to a double complement (average mode = 122 telocentrics). All clones also possessed human chromosomes, but fewer than the HeLa complement. The overall average mode was 27 human chromosomes; clones B and C had the highest number (20 to 45). The amount of human genetic material

Fig. 1. (A) Karyotype of mouse line 3T3-4(E) containing 69 telocentric chromosomes. (B) Karyotype of human line D98/AH2. (C) Karyotype of hybrid HLE-cl-C. Thirty-eight chromosomes (top three rows) have been classified as human, and 137 (bottom seven rows) as mouse.

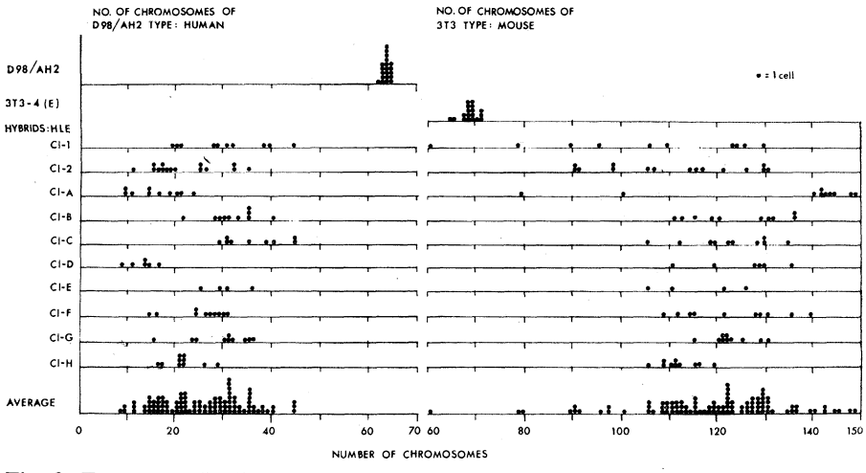

Fig. 2. Frequency distribution diagram of human type and mouse type chromosomes in ten clones of human-mouse hybrid HLE.

per cell is therefore considerably higher than in the hybrids described previously (*2*, *4*); but because of the duplication of the 3T3 complement, an unusual feature of this combination, the ratio of human to mouse genetic material is still only about 1:4. A karyotype of HLE-cl-C is shown in Fig. 1C; 38 chromosomes have been classified as human, and among them are members of every group.

The hybrid cells were very large. Growth was very slow at first, but within 2 months growth rates of the hybrids were of the same order as those of the parent lines, or slightly lower. On serial culture in standard medium, there was a slight tendency toward further loss of human chromsomes; clone C decreased in modal human chromosome number from 38 to 17 over the course of 100 cell generations. There was no concurrent loss of mouse chromosomes. Preferential loss of human chromosomes therefore occurs as in the human-mouse hybrids reported earlier (*2*, *4*), but considerably more slowly.

In an attempt to introduce a greater number of human chromosomes, a hybrid line was rehybridized in the following manner. A clone of HLE-C was transferred into medium containing BUDR at a concentration of 30 μg/ml. This selectively eliminates cells with the human chromosome bearing the thymidine kinase gene (*2*, *3*). Approximately 1 percent of the cells survived and grew into TK$^-$ colonies. One subline originating in this way had an average of 11 human chromosomes per cell and an undiminished number of 3T3 chromosomes. This population was mixed with D98/AH2 and the hybridization carried out in the usual manner, with the use of HAT to eliminate the parent cell types. Four clones of hybrid cells were isolated. All possessed from 15 to 45 human chromosomes, clone AB2 having the highest mode of 34 chromosomes. The number of chromosomes per cell was therefore not higher than that obtained after a single hybridization (HLE-C had a modal human chromosome number of 35).

Thus the selective elimination of human chromosomes occurring in human-mouse somatic cell hybrids reflects fundamental properties relative to the two species, while the culture history of the parent lines plays only a small part. To date the highest numbers of human chromosomes per hybrid cell occur in the HLE hybrids. Since (i) chromosome loss appears random, (ii) the average number of human chromosomes in some clones considerably exceeds 23, the number of haploid human chromosomes, and since most parent cell functions are expressed in somatic cell hybrids, most human gene products found in the human parent cell may be expected to be present in the HLE hybrids. For example, in contrast to the human-mouse hybrids reported earlier (*2*), which lack human gene products necessary for polio virus infection, the HLE hybrids are susceptible to this virus and may be used to identify human chromosomes bearing cellular genes necessary for the infectious process. In addition, study of these hybrids has revealed an interesting problem with respect to the control of ribosomal RNA synthesis, which will be reported elsewhere.

YUTAKA MATSUYA*
HOWARD GREEN
Department of Cell Biology, New York University School of Medicine, New York 10016

References and Notes

1. B. Ephrussi, in *Phenotypic Expression*, M. N. Goldstein, Ed. (Williams and Wilkins, Baltimore, 1967), pp. 40–45.
2. M. Weiss and H. Green, *Proc. Nat. Acad. Sci. U.S.* **58**, 1104 (1967).
3. Y. Matsuya, H. Green, C. Basilico, *Nature* **220**, 1199 (1968).
4. M. Weiss, B. Ephrussi, L. J. Scaletta, *Proc. Nat. Acad. Sci. U.S.* **59**, 1132 (1968).
5. W. R. Earle, *J. Nat. Cancer Inst.* **4**, 165 (1943).
6. G. J. Todaro and H. Green, *J. Cell Biol.* **17**, 299 (1963).
7. S. Kit, D. R. Dubbs, L. J. Piekarski, T. C. Hsu, *Exp. Cell Res.* **31**, 297 (1963).
8. W. Szybalski, E. H. Szybalska, G. Ragni, in *Analytical Cell Culture* (Nat. Cancer Inst. Monogr. 7, 1962), pp. 75–87.
9. J. W. Littlefield, *Science* **145**, 709 (1964).
10. K. H. Rothfels, E. B. Kupelwieser, R. C. Parker, in *Canadian Cancer Conference: Proceedings* (5th meeting at Honey Harbour) (Academic Press, New York, 1963), vol. 5, pp. 191–223.
11. S. M. Gartler, in *Decennial Review Conference on Cell, Tissue, and Organ Culture* (Nat. Cancer Inst. Monogr. 26, 1967), pp. 167–181.
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* Present address: Research Institute for Tuberculosis, Leprosy, and Cancer, Tohoku University, Sendai, Japan.

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Human Serum Inhibitor of C'1 Esterase: Identity with α_2-Neuraminoglycoprotein

Abstract. *A comparison of highly purified C'1 esterase inhibitor from human serum and α_2-neuraminoglycoprotein from human plasma by immunofusion, immunoelectrophoresis, and discgel electrophoresis showed them to be antigenically identical.*

The esterase activity (C'1 esterase) associated with the first component of human complement can be blocked by a protein inhibitor in normal human serum (*1*). This inhibitor (EI) is a heat- and acid-labile α_2-globulin which com-

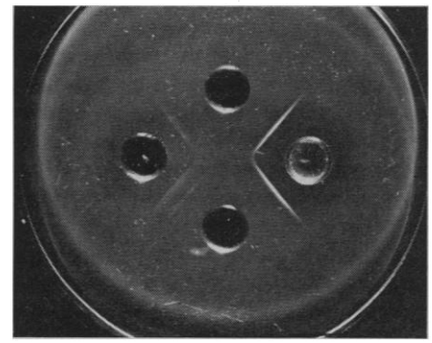

Fig. 1. Ouchterlony double diffusion experiment. Wells, cut in 0.5 percent (weight to volume) agarose, contained: top, C'1 esterase inhibitor; bottom, α_2-neuraminoglycoprotein; right, antiserum to C'1 esterase inhibitor; left, antiserum to whole human serum. Photographed after 26 hours of diffusion at room temperature.