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Human-Mouse Somatic Cell Hybrids with Single Human Chromosome (Group E): Link with Thymidine Kinase Activity

Abstract. *Mouse somatic cells lacking thymidine kinase were mixed in culture with human diploid cells lacking hypoxanthine guanine phosphoribosyl transferase, and hybrid cells were isolated and maintained in a selective medium containing hypoxanthine, aminopterin, and thymidine. The hybrid cells at the time of isolation had karyotypes consisting predominantly of mouse chromosomes but with one human chromosome, a submetacentric member of group E, apparently giving thymidine kinase to the hybrid cell. However, after long-term propagation in the selective medium this chromosome has been lost, although cells continue to show thymidine kinase activity as demonstrated by the incorporation of ^{3}H-thymidine into DNA in the hybrid cell. The hybrid cells have only mouse electrophoretic variants for glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and malate dehydrogenase, suggesting that the human genetic loci for these enzymes are not represented in the hybrid genome and may be unlinked to that for thymidine kinase.*

Weiss and Green have described human mouse hybrids containing a partial complement of human chromosomes (*1*). One of the human chromosomes was apparently associated with thymidine kinase activity, since this chromosome was present only in cells able to grow in medium containing hypoxanthine, aminopterin, and thymidine (HAT), which selects for the presence of the enzyme, and not in cells maintained in medium containing 5-bromodeoxyuridine (BUDR), which selects for cells with thymidine kinase deficiency.

We have mixed mouse somatic cells lacking thymidine kinase with human diploid cells lacking hypoxanthine guanine phosphoribosyl transferase and have isolated hybrid cells as a result of their ability to grow in selective medium which kills the mutant parent cells (*2*). The human parent cells were fibroblasts obtained by skin biopsy from a male with Lesch-Nyhan syndrome, and were isolated in this laboratory. They lack hypoxanthine guanine phosphoribosyl transferase (*3*), are resistant to 2 amino 6-mercaptopurine (10 μg/ml), and are unable to grow in medium containing HAT. These diploid cells have 46 chromosomes; chromosomes of groups E (Nos. 16 to 18) and F (Nos. 19 and 20) (*4*) are easily distinguished in morphology from chromosomes of the parental mouse cell.

The mouse parent, a subline of L-M mouse fibroblasts [LM (TK$^-$)] clone 1-D, was provided by Dr. M. Weiss. This clone of mouse fibroblasts was isolated by Kit *et al.* (*5*) and is resistant to BUDR (30 μg/ml) by virtue of its thymidine kinase deficiency. Clone 1-D has 52 (49 to 56) chromosomes with ten metacentric and submetacentric chromosomes, including the distinctive submetacentric marker D chromosome. The remaining chromosomes are telocentric (Table 1).

Hybrid cultures were initiated by mixing together 2×10^6 mouse and 2×10^6 human cells, in a 1-ml volume of glucose-free Hanks' solution. Ultraviolet-irradiated Sendai virus was added to one-half of the suspension of mixed cells to enhance contact between cells (*6*). The cell suspension that was not treated with virus and the Sendai-treated cell suspension were each plated into a 100-mm Falcon plastic petri dish, and the mixed populations were maintained in growth medium (minimum essential medium, 1 percent nonessential amino acids, 10 percent fetal calf serum) for 48 hours, at which time the medium was changed to HAT [growth medium + hypoxanthine ($1 \times 10^{-4}M$), aminopterin ($4 \times 10^{-7}M$), and thymidine ($1.6 \times 10^{-5}M$) + glycine ($3 \times 10^{-6}M$)]. Both mouse and human parental cells showed toxicity with cessation of growth in selective medium. After a latent period of 4 weeks HAT-resistant clones were first observed, and only in the petri dish containing ultraviolet-irradiated Sendai-treated cells. Twelve independent clones were isolated from this petri dish during the 6th week after mating. Eight clones have survived propagation in HAT for at least 6 months, and have been observed in regard to karyotype and enzyme characteristics.

Karyotypes of the hybrid cells during the course of propagation were prepared according to methods previously described (*7*). From earliest observations the hybrid cells had a karyotype consisting of almost the entire mouse genome with only one or two human chromosomes. Table 1 shows the characteristics of the karyotype of the mouse parent, clone 1-D, and of the hybrid cells close to the time of isolation. Four of the 21 hybrid cells analyzed had no apparent human chromosome. The remaining cells had included in their genome a chromosome morphologically similar to a submetacentric member of the human group E (Fig. 1). There were four cells which had, in addition to the previously described E group chromosome, one to two other chromosomes, morphologically different from those normally seen in clone 1-D. These presumably human chromosomes were, with one exception, also submetacentric members of group E. That the total numbers of chromosomes in the hybrid cells were essentially the same as those of the mouse parent is attributable to the loss in the hybrid cells of a mouse telocentric chromosome.

The association of the human submetacentric E group chromosome with thymidine kinase activity was suggested by our inability to find this chromosome in 116 hybrid cells presumably lacking thymidine kinase since they had survived transfer to media containing BUDR. Because the hybrid clones were isolated by virtue of their ability to grow in HAT, we assumed that these clones were thymidine kinase positive.

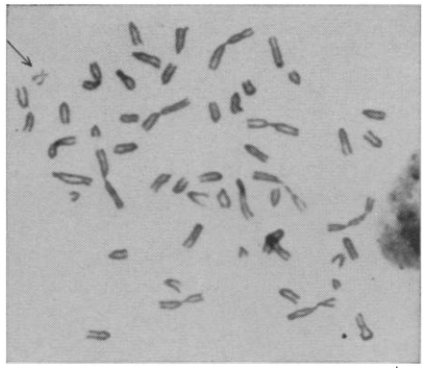

Fig. 1. Metaphase from hybrid cell showing nearly all of the expected mouse chromosomes with the single human submetacentric group E chromosome indicated by arrow.

To confirm the presence of thymidine kinase in the hybrid cell, clone 1-D and hybrid cells were exposed to ^{3}H-thymidine and autoradiographs were obtained. Cells growing in monolayer on microscopic slides in 100-ml Falcon plastic petri dishes were incubated in medium containing ^{3}H-thymidine (6.7 c/mmole, with a final concentration of 1 μc/ml of medium) for 6 hours. The slides were washed in saline, fixed in 3 : 1 methanol–acetic acid fixative, and flame dried. The preparations were stained with aceto orcein, dipped in Kodak NTB-2 emulsion, exposed for 1 week, and developed. The mouse parent cells lacking thymidine kinase were unable to incorporate ^{3}H-thymidine into their nuclei, while 74 to 92 percent of the hybrid cells were well labeled, indicating thymidine kinase activity.

Table 1. Characteristics of the karyotype of clone 1-D and hybrid cells.

Item	Clone 1-D	Hybrid (early)*	Hybrid (late)†
No. of cells analyzed	29	21	22
No. of chromosomes			
Range	49–56	48–54	48–53
Mode	52	53	50
Mean	52.45	52.05	50.50
Metacentric			
Range	4–8	5–10	4–8
Mode(s)	6	6, 7	7
Mean	6.55	6.86	6.31
Submetacentric			
Range	1–6	1–5	2–5
Mode	3	3	2
Mean	3.24	2.95	2.86
Human			
Range		0–3	
Mode		1	
Mean		1.15	
Telocentric			
Range	38–46	37–43	39–44
Mode(s)	43, 44	41, 43	41
Mean	42.52	41.14	41.32

* Cells from first to fourth subcultures. † Cells after 30th subculture whose karyotype does not include human submetacentric marker.

After approximately 4 months of serial transfers in culture, the karyotype of the hybrid cells had evolved so that in some of the hybrid clones the human submetacentric chromosome was no longer present. Table 1, column 3, shows the characteristics of the karyotype of these hybrid cells lacking the E group chromosome. The chromosome number had decreased, reflecting the loss of human chromosome(s). At this time, these cells were growing vigorously in HAT, achieving confluence weekly following a 1 : 100 split. When these cells were transferred to medium containing BUDR no growth was observed for at least 10 days, after which, in several of the clones, nonclonal but significant populations of BUDR-resistant cells began to overtake the cultures. Autoradiographs from these hybrid clones growing in HAT show that these cells, despite the lack of the submetacentric group E chromosome, maintained the ability to incorporate ^{3}H-thymidine, indicating the continued presence of thymidine kinase activity.

The only human genetic material which gives the hybrid cell any advantage in the selective medium is that providing thymidine kinase for the cell, so that it is not surprising that a relevant chromosome is retained. There may be more than one genetic locus involved in conferring thymidine kinase activity to the hybrid cell, but certainly the submetacentric E chromosome contributes one of them. During the first 3 months in culture the submetacentric chromosome was found only in medium selecting for the presence of thymidine kinase and was absent in medium selecting against cells with this enzyme. We believe that the human chromosome in our hybrid cells is the same one described originally by Weiss and Green (*1*) as a member of group C but which is now interpreted by them to represent also a submetacentric member of the E chromosome group (*8*).

The earliest karyotypes of these hybrid cells obtained at the time of clonal isolation consistently showed a small population of cells growing in selective medium which did not have the human group E chromosome. In subsequent subcultures the proportion of cells lacking the submetacentric chromosome increased so that karyotypes from most recent subcultures of some hybrid clones growing in HAT do not include this chromosome in any cell. The absence of the relevant chromosome from cells growing vigorously in HAT and which are unable to survive in BUDR suggests that the pertinent genetic material may have been translocated to a mouse chromosome and therefore incorporated into the mouse genome. We have no cytological evidence for a translocation chromosome but the presence of thymidine kinase in these recent cultures makes such an event likely.

Thymidine kinase activity has been induced in lines of the mouse parent cells by Kit *et al.* (*9*) through the introduction of vaccinia virus. Presumably the enzyme was made according to specifications in the viral genome. Despite numerous attempts to isolate cells which revert to the thymidine kinase positive state and extensive use of this particular mouse clone, there has not been a single instance of spontaneous reversion to wild-type enzyme function reported (*5*, *9*). The absence of activity of the enzyme in these mouse cells is therefore assumed to be due to deletion of the structural locus. Confirmation that the thymidine kinase in the hybrid cell has been specified by the human genome awaits elucidation of the nature of the thymidine kinase in the cell.

Shortly after isolation of the hybrid cells sonicated cell extracts of each hybrid clone were analyzed, according to methods previously described (*7*, *10*) for enzymes whose electrophoretic mobility differed in the mouse and human parental cells. The hybrid cells have only mouse electrophoretic variants for glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and malate dehydrogenase, suggesting that the human genetic loci for these enzymes are not represented in the hybrid genome and are therefore unlinked with thymidine kinase activity.

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