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Properties of Somatic Cell Hybrids Between Mouse Cells and Simian Virus 40-Transformed Rat Cells

J. VAN DER NOORDAA, ANNE VAN HAAGEN, J. M. M. WALBOOMERS, AND H. VAN SOMEREN[1]

Laboratorium voor de Gezondheidsleer, University of Amsterdam, Mauritskade 57, Amsterdam, The Netherlands

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Hybrids between mouse cells and simian virus 40 (SV40)-transformed rat cells were made, and their properties and chromosome constitution were investigated over many generations. Their hybrid nature was confirmed by enzyme studies. During a period of 1 year a loss of 10 to 20% of the total number of chromosomes was observed. The SV40 tumor antigen was present and remained present in the hybrids. The parental and hybrid cells were studied for agglutination with concanavalin A, for growth in soft agar, and for serum requirement. These growth and surface characteristics of the transformed cells appeared in the hybrids.

Several hybrid cell lines originating from the fusion of normal and virus-transformed cells have been described. Defendi et al. (4) have studied the properties of hybrids between normal mouse cells and mouse cells transformed by polyoma virus. The hybrid cells contained the polyoma virus-specific tumor and transplantation antigens, indicating the persistence of the viral genome in the hybrid cell.

Hybrids between mouse cells and polyoma-transformed hamster cells were shown by Basilico and Wang (1) to contain the viral genome and appeared to have a number of properties of the transformed cells. Hybrids between mouse cells and simian virus 40 (SV40)-transformed human cells were described by Weiss (13). The SV40-specific tumor (T) antigen was present in the nuclei of the hybrid cells. This type of interspecific hybrid undergoes a rapid loss of human chromosomes and therefore was considered suited to a study of the effect of chromosome loss on the presence of the SV40 viral genome. A positive correlation was found between the loss of human chromosomes and the loss of T antigen from the hybrid cells, suggesting integration of the SV40 genome into the chromosomes of the transformed cell.

In our study we report on the properties of hybrid cells between SV40-transformed rat cells and normal mouse cells. According to Weiss and Ephrussi (14), rat-mouse hybrids preferentially lose rat chromosomes, and therefore this type of hybrid was chosen to study the effect of chromosome loss on the properties of the hybrid cell.

MATERIALS AND METHODS

Rat cells. Cultures of primary rat cells derived from the brains of newborn BN/BI rats were inoculated with SV40 (strain VA 45-54) at a multiplicity of infection of about 10. A line of transformed cells was initiated from a focus of transformed cells. This line will be referred to as rat-SV40 cells. All cells of this line contained the SV40 T antigen. No infectious virus could be recovered from the transformed cells after either co-cultivation or fusion with monkey kidney cells (BSC-1 and CV1).

Mouse cells. A thymidine kinase-deficient derivative from 3T3 cells was kindly provided by D. Bootsma (Dept. of Cell Biology and Genetics, Medical Faculty, Rotterdam) and will be referred to as 3T3 TK$^-$.

Hybrid cells. The isolation of hybrid cells was performed by the half-selective system of Davidson and Ephrussi (3). The rat-SV40 (at passage level 13) and 3T3 TK$^-$ cells were mixed in a ratio of 1:1,000 and fused by Sendai virus (10) inactivated with beta-propiolactone. After 24 hr of incubation at 37 C, the medium was replaced by Littlefield's selective medium (7). This medium consists of standard medium supplemented with 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine (HAT medium). After two weeks of further incubation, two types of colonies were observed: colonies with the morphology of rat-SV40 cells and colonies with a morphology different from the rat-SV40 cells. Three of the latter colonies were isolated with the aid of a Pasteur capillary pipette, and one of these three identical-looking colonies (H) was used for further study.

No macroscopically visible colonies of 3T3 TK$^-$ cells were present at that time, although small groups

[1] Present address: Medical Biological Laboratory T.N.O., Rijswijk, and Laboratory for Molecular Genetics, University of Leiden.

or single cells with the morphology of 3T3 cells could be found.

From colony H, four subclones were derived for further experiments; these lines will be referred to as H2, H3, H8, and H10.

Culture conditions. All cultures were maintained in Eagle basal medium supplemented with 10% calf serum. Parental and hybrid lines were subcultured twice a week.

Enzyme studies. Enzyme electrophoresis was performed on cellulose acetate gel (9) with cell lysates of parental and hybrid lines. The following enzymes were tested: lactate dehydrogenase A and B (LDH A and B), 6-phosphogluconate dehydrogenase (6PGD), and indophenol oxidase (IPO).

SV40 T antigen. The SV40 T antigen was detected by the indirect immunofluorescence test (12). For all tests, serum from the same pool was used. The serum was obtained from hamsters bearing SV40-induced tumors. Fluorescein-labeled antihamster globulin was purchased from Nordic Pharmaceuticals and Diagnostics.

Agglutination by concanavalin A. Cultures were washed two times with Ca- and Mg-free phosphate-buffered saline (PBS) and dispersed with 0.02% ethylenediaminetetraacetic acid at 37 C. The dispersed cells were then washed twice with PBS free from Ca^{2+} and Mg^{2+}. To study the effect of trypsin on agglutination, cells were treated for 2 min with 0.05% trypsin, centrifuged, washed two times with PBS, and resuspended. The agglutination reaction was performed in 25-mm petri dishes (Falcon) with 0.5 ml of cell suspension (10^6 to 3×10^6 cells/ml) and 0.5 ml of a solution containing 5, 25, 50, or 125 μg of concanavalin A. Concanavalin A (Serva, Heidelberg) was dissolved in saline, *p*H 7. After gently shaking for 15 min at 25 C, the degree of agglutination was scored by two investigators as the percentage of agglutinated cells.

Serum requirement. To determine the serum requirement, cultures were fed with medium supplemented with 1, 5, and 10% serum. An inoculum of 2×10^4 cells was seeded in 60-mm petri dishes (Falcon), and the cells were counted after 1, 2, and 3 days.

Ability to grow in soft agar. Appropriate dilutions of cells in 2 ml of 0.34% agar medium were planted on a base layer of 5 ml of 0.5% agar medium in small flasks (Falcon); the number of colonies was counted after 2 weeks.

RESULTS

Enzyme and chromosome studies. The zymogram patterns of the four hybrid lines for LDH, 6PGD, and IPO showed intermediate bands; Fig. 1 represents the results for one of the hybrids. These findings showed the hybrid nature

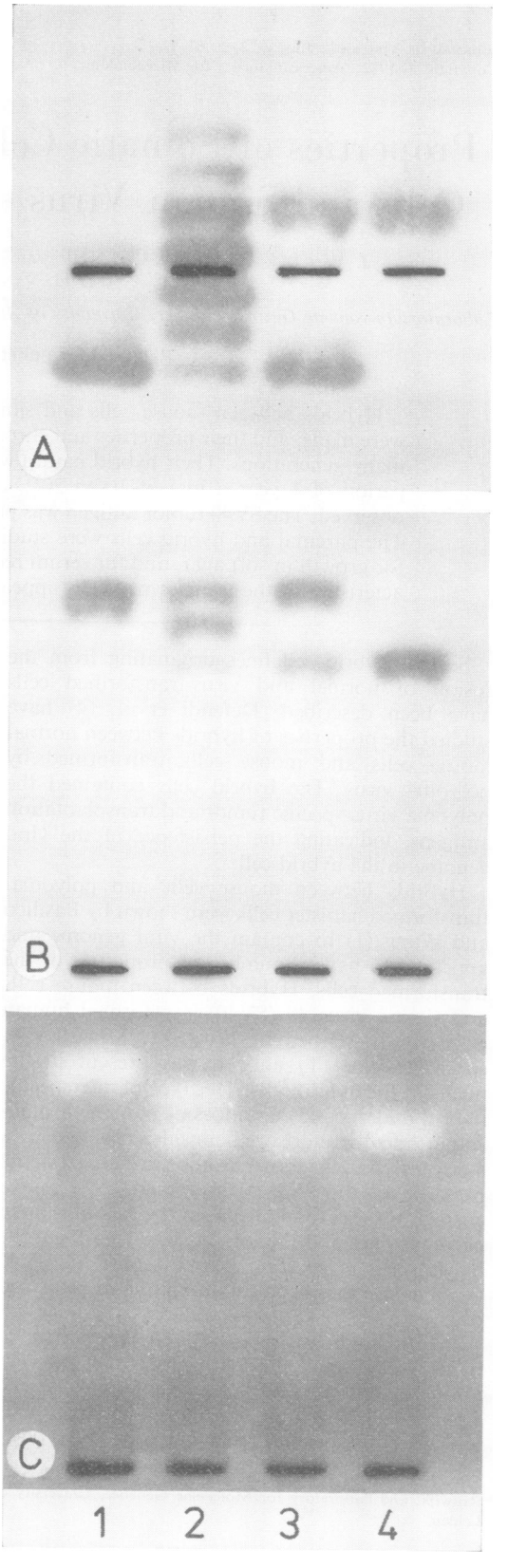

FIG. 1. *Zymograms of lactate dehydrogenase (A), 6-phosphogluconate dehydrogenase (B), and indophenol oxidase (C) showing rat-SV40 (1), hybrid H3 (2), artificial mixture of rat-SV40 and 3T3 TK⁻ (3), and 3T3 TK⁻ (4) patterns.*

of the lines H2, H3, H8, and H10 at passage 14. Starting at passage 20, most of our studies were performed on cells cultured in standard medium and on cells cultured in HAT medium. At passage 45 the hybrid cells were tested again; their hybrid nature had been retained for the tested enzymes in both media used.

Figures 2 and 3 show the results of the chromosomal studies of the parental mouse 3T3 TK$^-$ cells and rat-SV40 cells. The modal number of chromosomes of the 3T3 TK$^-$ cells is 63; they are all telocentric or acrocentric. The rat-SV40 cells had a modal number of 42 chromosomes, of which about 24 were telocentric or acrocentric and about 18 were metacentric or submetacentric. The number and distribution of the four hybrid cell lines are shown in Table 1. When tested at about the 12th subculture after cloning, the cells of the four hybrid lines contained 70 to 80 telocentric or acrocentric chromosomes and 11 to 16 metacentrics. Upon further subculturing, a small

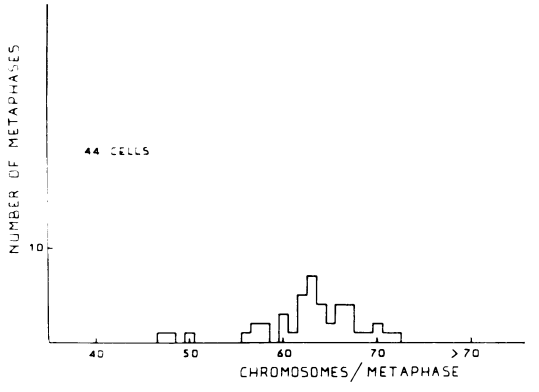

FIG. 2. *Histogram of chromosomes/metaphase against number of metaphases of 3T3 TK$^-$ cells.*

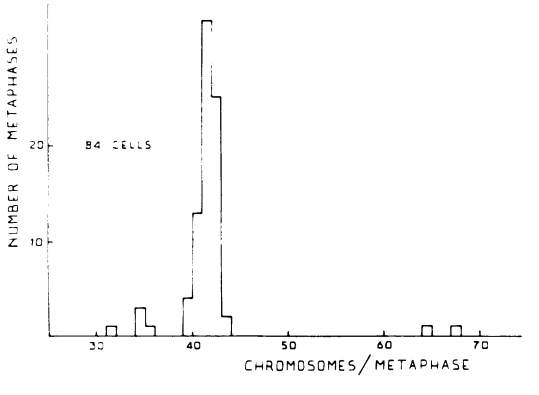

FIG. 3. *Histogram of chromosomes/metaphase against number of metaphases of rat-SV40 cells.*

loss of telocentric or acrocentric chromosomes and a minor change in the number of metacentric or submetacentric chromosomes was observed. It remains open to question whether rat or mouse chromosomes were lost.

Infectious virus and SV40 T antigen. The rat-SV40 and hybrid cells did not shed infectious SV40, nor was it possible to induce the production of SV40 by means of fusion with monkey kidney cells. The hybrid cells cultured in standard medium and HAT medium were first tested for the presence of the SV40 T antigen at about the 15th subculture. All cells from the four hybrid lines contained the SV40 T antigen. The type of fluorescence staining was similar to that of the rat-SV40 cells. Every fifth subculture of the hybrid cells was investigated for T antigen; they remained positive as far as tested, which includes the 90th subculture of the lines H2 and H8 in both media. The lines H3 and H10 have not been systematically investigated for T antigen beyond the 40th subculture.

Agglutination. The results of agglutination by concanavalin A with and without trypsin treatment of the parental and hybrid cells are shown in Table 2. Without trypsin treatment, agglutination of 3T3 TK$^-$ cells occurred after addition of 25, 50, and 125 μg of cancanavalin A. With 5 μg, no agglutination of 3T3 TK$^-$ cells was observed; at that concentration, 50% of the rat-SV40 and 25% of the hybrid cells had agglutinated after 15 min. With trypsin treatment, an increased agglutination of 3T3 TK$^-$ and hybrid cells was found. The degree of agglutination of the rat-SV40 cells had not changed.

Growth in soft agar. Parental and hybrid cells were tested for their ability to form colonies in soft agar; the results are shown in Table 3. The 3T3 TK$^-$ cells formed no colonies at all; about 20% of the transformed cells gave rise to colonies as did 1 to 4% of the hybrid cells.

The reduction of the efficiency of plating (EOP) in soft agar seems specific since the EOP in fluid medium of both rat-SV40 and hybrid cells was approximately 80%.

Serum requirement. The serum requirement of the hybrid lines was compared to that of the two parental lines. Figure 4 contrasts the effect of serum concentration on the growth rate of rat-SV40 and 3T3 TK$^-$ cells. The transformed cells grew at nearly the same rate in 10, 5, and 1% serum concentration. No increase in the number of 3T3 TK$^-$ cells was observed at the 1% serum concentration, and with a concentration of 5% serum the growth rate was still lower than with 10% serum. Figure 5 shows the response of the hybrid cells to serum concentration. Since all four

TABLE 1. *Number and distribution of chromosomes of the hybrid cells*[a]

Cell line	Subculture level	Standard medium			HAT medium[b]		
		Total no. of chromosomes	Telocentric/acrocentric	Metacentric/submetacentric	Total no. of chromosomes	Telocentric/acrocentric	Metacentric/submetacentric
Hybrid H2	12	96.4 (89–98)	80.6	15.7	NT[c]	NT	NT
	24	88.9 (84–97)	73.3	13.6	90.5 (77–100)	74.9	13.8
	62	80.4 (52–110)	65.7	12.7	83.0 (71–94)	66.8	14.6
	87	83.9 (64–88)	69.4	13.8	82.3 (53–134)	66.3	15.2
Hybrid H3	11	85.3 (65–96)	69.1	15.6	NT	NT	NT
	23	75.3 (59–102)	58.7	15.5	78.0 (55–92)	60.1	16.7
	39	69.6 (54–83)	53.8	14.5	68.8 (52–85)	51.8	16.0
	72	74.3 (60–85)	62.5	10.3	NT	NT	NT
Hybrid H8	11	95.8 (82–105)	80.3	15.3	NT	NT	NT
	26	87.0 (78–94)	71.2	14.2	88.2 (79–94)	71.9	14.9
	70	73.9 (60–83)	59.8	13.8	77.0 (59–89)	58.5	17.2
	89	78.0 (68–86)	60.9	16.3	74.7 (62–138)	55.0	19.2
Hybrid H10	12	92.2 (88–103)	77.4	14.4	NT	NT	NT
	19	90.4 (83–96)	75.6	13.5	88.5 (82–94)	74.6	12.9
	26	89.4 (83–98)	75.3	12.9	86.9 (73–94)	74.2	11.6
	42	75.3 (51–89)	61.1	13.3	78.1 (68–92)	64.3	12.9

[a] The number of chromosomes represents the mean value of 20 metaphases. The figures in parentheses denote the range of chromosomal numbers. The difference between the sum of telocentric/acrocentric and metacentric/submetacentric chromosomes and the total number is caused by chromosomes that cannot be identified.

[b] See Materials and Methods.

[c] NT = not tested.

TABLE 2. *Agglutination by concanavalin A of parental and hybrid cells*

Cell line	Subculture level	Trypsin treatment	Concanavalin A added (μg)			
			5	25	50	125
3T3 TK$^-$		Without	0[a]	1+	2+	2–3+
Rat-SV40	42	Without	2+	3–4+	4+	4+
Hybrid H3	38	Without	1+	2+	3+	4+
3T3 TK$^-$		With	0	3+	3–4+	4+
Rat-SV40	42	With	2+	3–4+	4+	4+
Hybrid H3	38	With	2+	3–4+	4+	4+

[a] 0 = No agglutination; 1+ = 25% of the cells agglutinated; 2+ = 50%; 3+ = 75%; 4+ = 100% agglutinated.

hybrid lines had about the same dependence on serum, the results with only one of them are given. Although the hybrid cells had a reduced growth capacity in 1% serum, there still was an increase in the number of cells.

DISCUSSION

The results of the chromosomal studies of the hybrid cells show that over a period of about a year only 10 to 20% of the chromosomes has been lost. The continuous presence of the three enzyme markers also indicates the stability of the hybrids. During this period of about a year, the cells were subcultured twice a week. The small loss of chromosomes is in agreement with the findings of Weiss and Ephrussi (14). In the study of rat-mouse hybrids, 5 to 10% of the chromosomes was lost over a period of 8 months; the percent loss of rat marker (metacentric) chromosomes was greater than that of the total number of chromo-

TABLE 3. *Efficiency of colony formation in agar of parental and hybrid cells*

Cell line	Subculture level	Colony-forming cells (%)[a]
3T3 TK$^-$		0
Rat-SV40	78	20.0
Hybrid H2	33	1.0
	78	1.2
Hybrid H8	35	3.2
	80	4.0

[a] Percentage is calculated from cell inocula of 2,000.

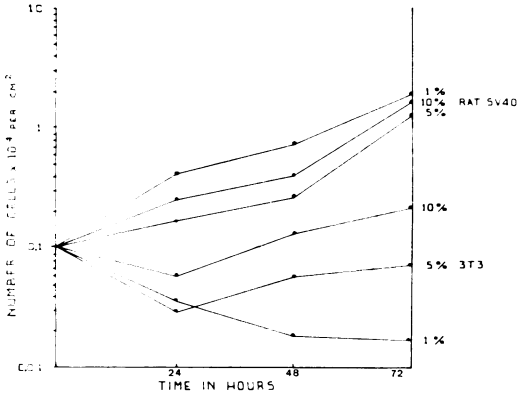

FIG. 4. *The growth of 3T3 TK$^-$ and rat-SV40 cells as a function of the serum concentration.*

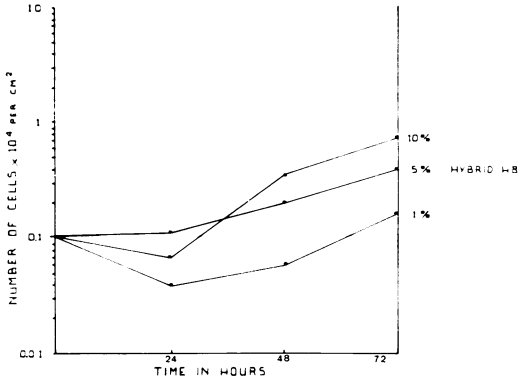

FIG. 5. *Growth of hybrid cells as a function of the serum concentration.*

somes, suggesting a preferential loss of rat chromosomes. In our studies, no preferential loss of metacentrics was observed, and most of the chromosomal loss occurred among the telocentric and acrocentric chromosomes. Further subculturing of the hybrid cells is necessary to obtain further loss of chromosomes; this will perhaps be enhanced by changing the experimental conditions.

Because identification of chromosomes remains difficult, the use of more enzyme markers might be valuable for studying the loss of parental genomes from the hybrid cells.

All properties of the SV40-transformed rat cells appear in the hybrid cells. The SV40 T antigen has been studied over many cell generations, and no loss of T antigen from the hybrid cells was observed. This is not surprising in view of the findings of Weiss (13) who studied the loss of T antigen from hybrids between mouse and SV40-transformed human cells. Cells became negative for T antigen only after most of the human chromosomes were lost. Since in our study only a very limited loss of chromosomes has occurred, the continuous presence of T antigen in the hybrid cells is not unexpected.

During further subculturing, the hybrid cells will be studied for the presence of T antigen and the number of rat chromosomes. The presence of T antigen indicates that the hybrid cells contain at least an early function of the viral genome. Since no infectious virus could be recovered it is not known whether the whole virus genome is present.

The fact that the SV40-transformed parent cells did not yield infectious virus was considered advantageous. The presence of infectious virus in our system might, after superinfection of negative cells, lead to the induction of T antigen or to transformation of the mouse component of hybrid cells.

The altered structural organization of the surface membrane as demonstrated by agglutination with concanavalin A was found in SV40-transformed cells (6). The degree of agglutination of our hybrid cells suggests that the surface properties of the transformed cells are at least partially present in the hybrid cells. Trypsin treatment of the hybrid cells resulted in an enhanced agglutination by concanavalin A. It has been shown that trypsin treatment of normal cells leads to an increased agglutination by glycoproteins (2, 6, 11). From this it seems reasonable to suppose that the hybrid cells also have surface properties of normal cells. It is not clear whether the surface properties of the hybrid cells result from suppression of the transformed phenotype by the normal mouse genome.

In addition, we have studied the growth characteristics (5) of the hybrid cells in comparison to those of the parental cells. All growth characteristics of the transformed cells were expressed in the hybrid cells, although to a lesser degree. This might enable us to determine whether the eventual

changes of the transformed phenotype of the hybrid are related to loss of chromosomes and enzyme markers. It has been shown by Marin and Littlefield (8) that one of the growth characteristics of polyoma-transformed hamster cells, growth in soft agar, was reduced after loss of chromosomes. As far as studied, no changes in growth characteristics have occurred in our hybrid cells, which is not unexpected in view of the small loss of chromosomes. Future studies will be directed toward relating loss of chromosomes and enzyme markers to properties of the hybrid cells.

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