*LOSS OF T-ANTIGEN FROM SOMATIC HYBRIDS BETWEEN MOUSE CELLS AND SV40-TRANSFORMED HUMAN CELLS**

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Long after infection with simian virus 40 (SV40), cells which have been transformed, although they do not shed infectious virus, can be shown to produce virus-induced antigens: the nuclear T-antigen or induced complement-fixing antigen (ICFA)[1] and a new transplantation antigen.[2] That such transformed cells do contain the complete virus genome has recently been demonstrated by reactivation of SV40 which was obtained by using inactivated Sendai virus to produce fusion of transformed cells with indicator (green monkey kidney) cells.[3, 4] However, the nature of the relationship of the SV40 genome to the transformed cell remains to be determined.

Among the most obvious possibilities are: (1) integration of the viral genome in that of the transformed cell, or (2) a relationship analogous to that described in *Paramecium* where the maintenance of a plasmid (kappa) requires the presence of definite chromosomal genes. It appeared to us that, in principle, a choice between these alternative mechanisms could be made by studying somatic hybrids which result from the fusion of mouse cells with SV40-transformed human cells, and which lose preferentially the human chromosomes.

It will be recalled that somatic hybrids resulting from the "cross" of diploid human fibroblasts with cells of a permanent line of mouse origin undergo rapid and extensive loss of human chromosomes.[5] In these human-mouse hybrid cells, nearly all of the chromosomes can be identified as to species of origin. The decline in number of human chromosomes occurs over a period of several months (about 100 cell generations), and in some cases results in loss of all detectable human chromosomes with no decline in the number of mouse chromosomes.

These observations suggested that somatic hybrids between mouse cells and SV40-transformed human cells would permit the correlation of the presence of virus-induced antigens with the number and kinds of human chromosomes retained in the hybrid cells. Moreover, since it is known that both human and mouse cells can be transformed by SV40, loss of virus-induced antigens (and/or loss of the SV40 genome) from hybrid cells, if it occurred, could not be ascribed to loss of cellular genes of one species required for maintenance or expression of the virus.

Two human × mouse crosses were undertaken for these experiments. In both cases, lines of mouse cells which had never been exposed to SV40 were crossed with human lines transformed by the virus. The first cross involved the thymidine kinase-deficient mouse line LM(TK$^-$)*cl* 1 D[6] and the "pre-crisis" clone SV-SD-C, obtained by Todaro, Wolman, and Green[7] by transformation of human diploid skin fibroblasts with SV40. A single hybrid colony (designated HM-SV) was isolated by using the "half-selective system" of Davidson and

Ephrussi.[8] The mixed cultures and the hybrid cells were maintained in medium containing anti-SV40 antiserum.

In the second cross, normal diploid fibroblasts obtained from a newborn mouse carrying the T-6 translocation were crossed with cells of an 8-azaguanine-resistant subclone derived from WI-18-VA-2, an SV40-transformed human line which has been free of infectious virus for several years.[9] The hybrids (designated VT) were selected as above, and several hybrid colonies of independent origin were isolated.

The T-antigen assays were performed as follows: Cells were seeded on cover slips at low density and allowed to grow for four to eight days. At this time, numerous discrete colonies were present. The cover slips were rinsed, fixed for 10 minutes (7 parts methanol:3 acetone) in the cold, incubated in fluorescein-labeled antiserum (Flow Laboratories), rinsed again, and mounted in elvanol. By this method, it was possible to identify individual colonies as positive or negative.

All hybrid cultures were identified karyologically about 20 cell generations after their formation. At this time, the HM-SV hybrids contained all of the expected *cl* 1 D chromosomes (43 telocentrics and 9 long metacentrics, including the characteristic "D" chromosome [10]) and up to 10 human chromosomes. The evolution of the karyotype of this hybrid line has been followed for more than 120 generations. During this time, the number of human chromosomes has decreased; some clones have been isolated which contain no detectable human chromosomes, while others contain five or more human chromosomes.

The hybrids of the VT series, when first examined, contained 3–30 human chromosomes as well as 60–80 typical mouse telocentrics, among them 2–4 T6 chromosomes.[11] Two clones, VT no. 2 and VT no. 7, have been followed for 100 generations, during which time the number of human chromosomes has decreased. However, as yet no clones have been isolated which have lost all human chromosomes.

T-antigen assays have been performed on all four of the parental lines and on the various hybrid populations, as described above. Neither of the mouse parental lines contained any T-antigen positive cells, while in both SV40-transformed human lines, every cell was found to contain T-antigen. Analysis of the VT hybrids, performed after 50–55 generations, indicated that every hybrid cell contained T-antigen. These assays were repeated every 10-20 generations, and after 100 generations, VT no. 7 was found to contain a small proportion of cells giving rise to negative colonies (Table 1). This population has been cloned, and an attempt is being made to isolate negative clones in pure cultures. Their karyotypes will be compared with those of positive clones.

The first T-antigen assay performed on the HM-SV hybrids was done after about 30 generations, and at this time, the hybrid population was mixed, that is, it contained cells giving rise to both positive and negative colonies (Table 1). That both cell types are viable has been demonstrated by the isolation of pure subclones of positive and negative cells. Moreover, it has been possible to isolate negative subclones from positive ones (Table 1, subclone 6–3 derived from clone 6), a fact which provides clear evidence that T-antigen negative cells do arise from positive ones.

TABLE 1. *SV40 ICFA and number of human chromosomes in somatic hybrids between mouse cells and SV40-transformed human cells.**

Population and Number of Generations Grown		Mean no. (and range) of human chromosomes	SV40 ICFA
VT no. 2	55	12.6 (10–15)	Positive
VT no. 7	50	8.4 (3–23)	Positive
VT no. 2	95	11.1 (9–22)	Positive
VT no. 7	100	10.1 (2–17)	Mixed
HM-SV	30	5.2 (2–10)	Mixed
Clone 3a	60	2.9 (2–4)	Mixed
Clone 4	60	2.0 (2)	Positive
Clone 6	60	2.1 (1–3)	Positive
Clone 6-1	120	2.0 (2)	Positive
Clone 6-3	120	1.0 (1)	Negative
Clone 6-4	140	1.9 (1–2)	Positive
HM-SV	80	1.9 (1–4)	Mixed
Clone 1†	100	0 (0)	Negative
Clone 2†	100	0.4 (0–1)	Negative
Clone 3†	100	0 (0)	Negative

* All hybrid cultures, except those indicated by dagger superscripts, were maintained in selective medium containing hypoxanthine, aminopterin, and thymidine.[8] In this medium, all cells of the HM-SV series contain at least one human chromosome: that required for the synthesis of human thymidine kinase (see ref. 5).

† Grown in medium containing 5-bromodeoxyuridine, which selects against thymidine kinase.

Study of karyotypes of the HM-SV hybrids at 20 generations after their formation has shown that they contained a mean number of 5.2 human chromosomes. This number decreased to 1.9 after 80 generations of growth. The various subclones have contained 2.0–2.9 human chromosomes (5 T-antigen positive subclones) and 0–1.0 human chromosomes (5 T-antigen negative subclones). From these results, it would appear that T-antigen positive cells become negative only when all or nearly all of the human chromosomes have been lost. Comparative analysis of additional positive and negative subclones should indicate: (1) whether it is possible to obtain negative cells which retain a larger number of human chromosomes and (2) whether there is a particular human chromosome, the loss of which is invariably correlated with the conversion of cells from T-antigen positive to T-antigen negative.

Summing up, we have observed that T-antigen, present in the human parental cells before hybridization, (1) is expressed in the hybrid cells (this is in agreement with the findings of Defendi *et al.*[12] for the expression of polyoma T-antigen in hybrid cells) and that it (2) apparently is lost from the hybrid cells concurrently with the loss of human chromosomes. The latter observation suggests that there may be a relation between these two events.

As mentioned in the introduction, several hypotheses regarding the relationship of the virus genome to the cell it has transformed can be imagined. For example, the virus could exist as a free particle in the cytoplasm or in the nucleus of the transformed cell. If this were the case, we would not expect to observe loss of the viral antigen from the "highly segregated" hybrid cells if the remaining full mouse chromosome complement were adequate for the maintenance and expression of the viral genome.[13] It should be noted also that no cell line transformed by SV40 has been shown to undergo spontaneous loss of the virus genome.

A second hypothesis is that the viral genome is integrated into the chromosomes of the transformed cell. The observations reported above are compatible

with this hypothesis. It is, however, puzzling that the loss of T-antigen apparently requires the loss of nearly all human chromosomes and that some clones are positive even though they contain very few human chromosomes. Experiments underway are expected to show whether the loss of T-antigen can be correlated with the loss of a specific human chromosome.

More complex hypotheses include: (1) presence of the virus both as free particles and integrated into the host chromosomes and (2) presence of the virus within cellular organelles, such as mitochondria, in which it could be integrated into the mitochondrial DNA. If the latter hypotheses were true, loss of T-antigen might be due to loss of human genes required for maintenance of human mitochondria.

A more complete interpretation of our results will depend upon the outcome of further experiments. For example, we must isolate in pure culture T-antigen negative clones of the VT hybrids in order to determine: (1) that the negative cells are viable progeny of the positive cells, (2) whether loss of T-antigen occurs only when all human chromosomes are lost, and (3) whether and how the karyotypes of positive and negative cells differ. Finally, in order to conclude that loss of T-antigen is not due to loss of a (human) cellular gene required for maintenance of the viral genome, it will be necessary to obtain retransformation of negative cells.

Examination of other properties of the virus in the hybrid cells, including the SV40-induced transplantation antigen, and ability of the virus to be reactivated following Sendai-induced fusion with green monkey kidney cells[3] will show whether the T-antigen negative cells have lost the entire SV40-genome.

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[11] Since all of the hybrids examined contained nearly twice as many mouse chromosomes as expected, it would appear that they arose from fusion of tetraploid T_6 cells with diploid human cells.

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[13] It is known that, in general, mouse cells can be transformed by SV40 and that the transformants produce T-antigen. It must be pointed out, however, that SV40-transformation of *cl* 1 D, the mouse line used in these experiments, has not been directly demonstrated, even though we were able to observe T-antigen production following infection of cells of this clone with SV40.