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REVERSION IN VIRUS-TRANSFORMED CELLS

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Abstract—The hamster cell line BHK21 can be transformed by the Schmidt–Ruppin strain of Rous sarcoma (RSV-SR) and by polyoma viruses. Revertant cells that have apparently lost the virus genes can be obtained in both cases. These occur spontaneously in the case of RSV-SR transformed cells and following the induction of chromosome loss from near tetraploid BHK21 hybrid cells transformed by polyoma. In both cases the cells can be retransformed by the same virus that transformed them.

These results suggest that the continued presence of the viral genes in the cell is necessary for the expression of the transformed phenotype.

THE STUDY of virus-induced transformation has been facilitated by the use of cell lines with a high degree of autonomy *in vitro*. The BHK21/13 line of hamster fibroblasts behaves like normal cells in its response to factors which control cell growth and multiplication. These responses are altered in a characteristic way when the cells are transformed by polyoma virus (PV)[1] or Rous sarcoma virus (RSV).[2] The BHK21 cells and their transformed derivatives multiply rapidly and have high cloning efficiencies in platings for discrete colonies and in micromanipulative procedures involving the isolation of single cells. Transformed cells can be recognised by their disorderly heaped up growth on glass and also by their ability to form colonies in agar suspension culture.[3]

This paper describes the reversion of transformed cell properties in BHK21 cells transformed by RSV (an RNA-containing tumour virus) and PV, (a DNA-containing virus).

Transformation of BHK21 with the Schmidt–Ruppin strain of RSV

The Schmidt–Ruppin strain of RSV transforms a small proportion of BHK21 cells and confers on them the ability to form colonies in agar suspension culture. Cell lines established from the agar colonies are predominantly composed of transformed cells, but when they are cultured to produce discrete colonies on glass, a small proportion of the colonies are found with growth characteristics like the original BHK21 line, i.e. parallel orientation and little tendency to pile up. It was also found that when clones of the transformed cells were derived by careful micromanipulation of single transformed cells a proportion of their progeny had normal BHK21 cell properties.

Although the transformed cells do not release infectious virus they contain the virus genome and express a number of virus specific properties. The presence of the virus genome can be demonstrated by cultivating the transformed cells with chicken embryo fibroblasts. In such cultures infectious RSV appears in the medium, perhaps as a result of the RSV genome being transferred to a chicken embryo cell in which infectious virus can be completed, or alternatively as a result of spontaneous cell fusion resulting in a hamster–chicken cell hydbrid in which the virus can mature.

Similarly when the transformed hamster cells are inoculated into chickens, virus is formed and a chicken cell tumour develops. In those chickens the presence of circulating antibody capable of specifically neutralizing the Schmidt–Ruppin strain of RSV indicated that complete virus particles had been formed. The transformed cells also contain a complement fixing antigen common to viruses of the avian leukoses complex.

When the revertant clones were examined for these properties they were found to be negative and behaved like the original BHK21 cells. The properties of the transformed cells and the revertants are summarized in Table 1. It seems that the virus

TABLE 1. PROPERTIES OF ROUS SARCOMA VIRUS* TRANSFORMED BHK21/13 CELLS AND THEIR REVERTANTS

Clone	Colony morphology	Colony forming efficiency in agar (%)	Tumour induction in hamsters	Recovery of virus		Avian leukoses group specific antigen
				in vitro†	*in vivo*‡	
BHK21/13	///	<1	±	−	−	−
BHK21/SR§	*	65	++	+	+	+
BHK21/BHT‖	❀	50	++	+¶	not done	+
BHK21/SR/R1**	///	<1	±	−	−	−
BHK21/SR/R2**	///	<1	±	−	−	−

* Schmidt–Ruppin strain.
† Co-cultivation of clones with chicken embryo cells.
‡ Inoculation of clones intramuscularly into adult chickens.
§ Transformed by the Schmidt–Ruppin strain of RSV.
‖ Transformed by the Bryan high titre.
¶ Chicken embryo cells must be infected with helper virus (RAV 1).
** Revertant clones derived from BHK21/SR.

genome has been lost from the revertants and the simplest mechanism to explain this event is that the virus genome is incapable of replicating rapidly enough to keep pace with cell division. Eventually daughter cells are formed lacking an RSV genome and these appear as revertants. In platings of the transformed cell clones a spectrum of colonies develop with morphologies ranging from those that are completely transformed with random orientation and heaped up growth to colonies indistinguishable from BHK21. The intermediate colony types may represent cells possessing varying amount of RSV genomes i.e. the degree of morphological change is a function of viral gene dosage. By co-cultivating revertant cells with chicken embryo fibroblasts infected with RSV (Schmidt–Ruppin) to ensure a massive infection of the hamster cells a second cycle of transformation and reversion can be demonstrated.

Transformation of BHK21 with the Bryan high titre strain of RSV

The Schmidt–Ruppin strain of RSV used in these experiments produces low titres of infectious virus in chicken embryo cells and, as previously suggested, this property of the virus may account for the appearance of revertants in the rapidly growing BHK21 cells. If this model is correct it is possible that RSV strains capable of rapid replication to high titre are also capable of maintaining the transformed phenotype

in the cells they infect. This expectation was borne out in experiments with the Bryan high titre strain of RSV.[4] A small proportion of BHK21 cells were transformed and produced colonies in agar suspension following exposure to the virus. When grown on glass these cells are rounded or epithelioid like chicken embryo cells transformed by this strain of RSV. This character was maintained without reversion after many generations in tissue culture during a period of continuous cultivation extending for over a year. The transformed cells did not release virus but virus could be recovered from combined cultures of the transformed cells and chicken embryo fibroblasts previously infected with the helper virus RAV 1. Also BHK21 cells transformed by a variant of the Schmidt–Ruppin strain of RSV that grows to high titre in chicken embryo cells do not lose the virus genome or revert.[5]

Transformation and reversion of BHK21 *cells transformed by polyoma virus*

In contrast to the observations with RSV (Schmidt–Ruppin) BHK21 cells transformed by polyoma virus do not undergo spontaneous reversion. However, reversion has been obtained by inducing the transformed cells to lose chromosomes.[6] A near tetraploid hybrid line was obtained by co-cultivating variants of BHK21 cells lacking either acid pyrophosphorylase or thymidine kinase, i.e. cells resistant to 6-thioguanine and 5-bromodeoxyuridine respectively. The hybrids, which were sensitive to both drugs, were selected in medium containing aminopterin, hypoxanthine, thymidine and glycine. A polyoma virus-transformed hybrid clone when plated in medium containing 6-thioguanine yielded resistant variants with a frequency of 10^{-4}. Thioguanine resistance was associated with a reduction in the chromosome number of the

TABLE 2. PROPERTIES OF POLYOMA-TRANSFORMED BHK21/13 HYBRIDS AND THEIR REVERTANTS

Clone	Colony morphology	Colony forming efficiency in agar (%)	Modal chromosome number	Tumour inducing dose (50%)	Presence of induced T* and TR† antigens
BHK21/13	///	<1	44	$>5 \times 10^6$	−
BHK21/Py	*	40–80	44	$<10^2$	+
Hybrid/Py	*	39	75	$<10^2$	+
Hybrid/PyTG$^+$	* ///	28	52–54	$<10^2$	+
Hybrid/R_1TG$^+$	///	7	62–64	10^4	−
Hybrid/R_2TG$^+$	///	<0·1	57–58	$>10^6$	−

* T antigen = nuclear antigen demonstrated by complement fixation.
† TR antigen = "transplantation rejection" antigen demonstrated by reduced tumour producing capacity of cells possessing the antigen in hamsters previously injected with polyoma virus.

cells and a proportion of these clones grew like the untransformed BHK21. Marin and I[7] studied the properties of two such phenotypically reverted cell lines. They were found to be less tumorigenic than their hybrid precursor. They had also lost the polyoma specific complement fixing "T" antigen and transplantation rejection antigen (Table 2). These changes were probably due to the loss of the polyoma virus genome from the cell rather

than the loss of some host cell function required for their expression, since the revertants could be re-transformed when infected with polyoma virus. Although the revertant cells were less tumorigenic than their transformed precursors it is not possible to say that tumorigenicity is a virus *coded* property. It could be that virus transformation of a cell population favours the accumulation of tumorigenic variants. If these form a minority of the transformed cell population then revertants would have a higher probability of being derived from transformed, but non-tumorigenic members of the population.

These two examples of revertants from virus-transformed cells indicate that DNA and RNA viruses may form different types of interactions with the cells they transform although phenotypically they produce the same changes in the cells.

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