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## Abstract

The rescue of infectious virus from nonproducer BH RSV(-) cells by chick cellular DNA was attempted in order to investigate the functional state of endogenous and exogenous retroviral genes integrated within the cellular DNA. No infectious virus was rescued by transfection with DNAs of chick helper factor (chf)-negative chick embryo cells (CEC), chf-positive CEC or uninfected CEC producing endogenous Rous associated virus (RAV-0). On the other hand, infectious Rous viruses with the phenotype of RAV-0 and RAV-1 were rescued by transfection with DNAs of CEC which had been infected with RAV-0 and RAV-1. From these results, it seems that exogenous retroviral genes integrated in the cellular DNA are expressed rather easily by transfection while those present endogenously are not.

**KEYWORDS:** transfection, chick DNA, nonproducer Rous cell, virus rescue

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## Rescue of Infectious Virus from Nonproducer Rous Cells by Chick Cellular DNA

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The rescue of infectious virus from nonproducer BH RSV(−) cells by chick cellular DNA was attempted in order to investigate the functional state of endogenous and exogenous retroviral genes integrated within the cellular DNA. No infectious virus was rescued by transfection with DNAs of chick helper factor (*chf*)-negative chick embryo cells (CEC), *chf*-positive CEC or uninfected CEC producing endogenous Rous associated virus (RAV-0). On the other hand, infectious Rous viruses with the phenotype of RAV-0 and RAV-1 were rescued by transfection with DNAs of CEC which had been infected with RAV-0 and RAV-1. From these results, it seems that exogenous retroviral genes integrated in the cellular DNA are expressed rather easily by transfection while those present endogenously are not.

**Key words:** transfection, chick DNA, nonproducer Rous cell, virus rescue

All avian cells contain endogenous retroviral genes in their chromosomal DNA (1). In cells infected with exogenous avian retrovirus, viral genomes are integrated within the cellular DNA (1). Cooper and his associates examined, by transfection using chick embryo cells (CEC) as the recipient, the infectivity of endogenous and exogenous avian retroviral genes integrated within chick cells, and found that the endogenous ones lacked infectivity (2, 3).

BH RSV(−) cells, avian cells transformed by an envelope (*env*)-defective strain of Bryan high titer Rous sarcoma virus (BH RSV), produce no infectious virus (4). Infectious virus is rescued from them when

the *env*-gene product is supplied by superinfection with Rous associated virus (RAV) or by fusion with chick helper factor (*chf*)-positive CEC (4, 5). Consequently, rescue of infectious virus from BH RSV(−) cells is expected to be more efficient than that from CEC.

In the present study, using novel BH RSV(−) cells as the recipient for transfection, we investigated the difference in expression between endogenous and exogenous retroviral genes integrated within cellular DNA.

BH RSV(−)Q cells, BH RSV(−) Japanese quail cells transformed by BH RSV (6, 7), and *chf*-positive and -negative CEC were obtained from Dr. R. Friis (Giessen, West Germany). CHCC-OUI, CEC produc-

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ing endogenous RAV-0, was established in this laboratory as described previously (8). The specific pathogen-free C/O CEC were obtained from Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University, Kanonji, Japan. Standard viruses, RAV-1 (subgroup A), RAV-6 (subgroup B), RAV-7 (subgroup C), RAV-50 (subgroup D) and RAV-0 (subgroup E) were obtained from Dr. H. Bauer (Giessen, West Germany).

DNA was extracted from CEC by Marmur's method (9), with the following modifications: CEC on culture dishes were lysed in 10 mM Tris-HCl (pH 8.0) containing 0.5% sodium dodecyl sulphate, 50  $\mu\text{g}/\text{ml}$  Proteinase K (Serva, West Germany) and 1 mM ethylenediaminetetraacetic acid overnight at 37°C.

DNA (20  $\mu\text{g}$  per dish) was transfected into BH RSV(-)Q cells ( $2 \times 10^6$  cells per dish) by the calcium method (10). One to 2 weeks after transfection, culture supernatants were harvested and inoculated into C/O CEC to examine the presence of rescued infectious virus. The subgroup of rescued viruses was determined by virus interference test (11).

None of the culture supernatants of BH RSV(-)Q cells transfected with DNAs of *chf*-negative CEC, *chf*-positive CEC and uninfected CEC producing endogenous RAV-0, showed transforming activity to C/O CEC, indicating no infectious virus was rescued

(Table 1). On the other hand, infectious virus was detected in 3 out of 15 dishes of BH RSV(-)Q cells transfected with the DNA of CEC which had been infected with RAV-0. Infectious virus was also detected in 5 out of 15 dishes of BH RSV(-)Q cells transfected with the DNA of CEC which had been infected with RAV-1 (Table 1).

The virus rescued from BH RSV(-)Q cells by transfection with the DNA of RAV-0-infected CEC, interfered only with RAV-0, indicating that it was RSV(RAV-0), RSV with the envelope phenotype of RAV-0 (Table 2). The virus rescued by transfection with the DNA of RAV-1-infected CEC, interfered only with RAV-1, indicating that it was RSV(RAV-1), RSV with the enve-

Table 1 Rescue of infectious virus by transfection of BH RSV(-)Q cells with chick cellular DNA

Source of chick cellular DNA used for transfection	No. of infectious virus-positive dishes <sup>b</sup> /No. of dishes transfected
<i>Chf</i> -negative CEC <sup>a</sup>	0/15
<i>Chf</i> -positive CEC	0/20
Uninfected CEC producing endogenous RAV-0	0/20
CEC infected with RAV-0	3/15
CEC infected with RAV-1	5/15

a: CEC, chick embryo cells.

b: C/O CEC dishes were inoculated with the culture supernatant of BH RSV(-)Q cells transfected with chick cellular DNA, and observed for transformed foci. BH RSV(-)Q cell dishes were counted as positive if the culture supernatant induced transformed foci in C/O CEC.

Table 2 Interference between rescued virus and standard virus<sup>a</sup>

Virus used for challenge	Focus formation in cells infected with				
	RAV-1	RAV-6	RAV-7	RAV-50	RAV-0
Virus rescued by DNA from RAV-0-infected CEC	+	+	+	+	-
Virus rescued by DNA from RAV-1-infected CEC	-	+	+	+	+

a: C/O CEC were infected with each standard virus (RAV-1, RAV-6, RAV-7, RAV-50 and RAV-0), subcultured twice, challenged by rescued virus and observed for development of foci of transformed cells. No focus formation (-) indicates the establishment of interference between the challenge virus and the standard virus.

lope phenotype of RAV-1 (Table 2). It is thus evident that the genome, or the *env* gene at least, of exogenous RAV-0 and RAV-1 integrated within the cellular DNA is expressed by transfection into BH RSV(-)Q cells.

The results of the present study have shown that the endogenous RAV genome or the *env* gene is not expressed easily, while the exogenous RAV genome integrated within the cellular DNA is. As for the lack of infectivity in endogenous RAV-0 DNA, Cooper and his associates proposed that the endogenous retroviral genome could be linked to a cis-acting control element of cellular DNA that inhibits its transcription (2, 3). Our results using BH RSV(-)Q cells support this hypothesis, and further suggest that the exogenous retroviral genome could be integrated within a region of cellular DNA without a cis-acting control element.

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