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Isolation of Infectious Xenotropic Mouse Type C Virus by Transfection of a Heterologous Cell with DNA from a Transformed Mouse Cell

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An endogenous xenotropic type C virus has been isolated from a Kirsten sarcoma virus-transformed BALB/c mouse cell line by transfection of a mink fibroblast cell with the DNA from the transformed cells. The results indicate that transfection may be used as a technique to isolate this endogenous type C virus without the need to chemically induce the cell line containing the provirus prior to attempting to isolate the virus.

The original observations of the isolation of infectious type C proviral DNA leading to the recovery of replicating type C virus were made by Hill and Hillova (6, 7). This technique has been termed transfection (6, 7). These authors used the DNA from a Rous sarcoma virus-transformed non-virus producing rat cell (XC cell) to transfect a permissive chicken cell leading to the production of infectious Rous sarcoma virus in the permissive chicken cell. Subsequently, this observation has been confirmed and extended; infectious DNA has been obtained from a variety of cells which had been previously infected exogenously with either avian leukosis or avian sarcoma viruses, with an additional group of avian viruses, reticuloendotheliosis viruses (3, 9), or with a replicating mammalian type C virus, RD-114 (R. McAllister, personal communication). In addition, using 3T3 mouse cells as recipient cells, it has been shown that the defective Moloney sarcoma virus genome can be recovered from either HT-1 cells or D-243 cells by transfection (8). In these cases of successful transfection, the genome recovered has been that of a virus which had been used to exogenously infect the cell line from which the DNA was obtained. Thus, although infectious DNA has been recovered from both exogenously infected nonproducer cells (XC cells, HT-1 cells, and D-243 cells) and exogenously infected producer cells, in each case the DNA has been extracted from a cell which had been exogenously infected with a type C virus.

Recently, a group of type C viruses has been recognized which have the interesting property of being able to replicate in heterologous cells; however, they are unable to infect exogenously the cells in which these viruses normally reside. This class of type C viruses has been denoted as "xenotropic" viruses (10). Examples have been found in the avian system with the subgroup E avian leukosis virus (17), in several strains of mice (10), in cats (11), and most recently in baboons (2). By growth on heterologous cells, or selected cells of the same species, these xenotropic viruses have been isolated either after spontaneous release from the cells in which they normally reside or by induction of the cells in which they reside by a variety of chemical agents. Since the rate of spontaneous virus release varies widely in different cells (2, 10-12, 17) and chemical inducers vary in their efficacy in different cells (1, 12, 17), we have sought additional methods to isolate xenotropic viruses. We report here the isolation of an endogenous xenotropic virus from Kirsten sarcoma virus-transformed BALB/c cells by extracting the DNA from the cells and transfecting this naked DNA into a permissive heterologous cell, a mink fibroblast cell line (5). By transfection of the Ki-BALB DNA into the mink fibroblast cell, we have isolated a replicating murine type C virus which has the ability to infect a variety of heterologous cells but cannot replicate in either N-permissive or B-permissive mouse cells.

In initial experiments, the DNA from a Kirsten sarcoma virus-transformed nonproducer clone of BALB/c cells, Ki-BALB, was extracted and used to transfect the mink fibroblast cell line (CCL64) with the calcium phosphate method of Graham and Van Der Eb (4). The results of such a transfection are shown in Table 1. The initial method of detection of the successful transfection by the type C proviral DNA was the assay for viral reverse transcriptase in

TABLE 1. *Transfection of mink cells with Ki-BALB cellular DNA*[a]

Expt 1		Expt 2		
Days after treatment	[^{3}H]TMP incorporated (pmol)	Culture no.	Foci	Viral polymerase
3	<0.03	1	+	+
7	<0.03	2	−	+
13	0.3	3	+	+
20	14.2	4	−	+
28	18.1	5	−	+
35	16.5	6	−	+
		7	+	+
		8	+	+
		9	−	+
		10	−	+
		11	−	−
		12	+	+
		13-16[b]	−	−

[a] The DNA from Ki-BALB nonproducer cells was extracted with 1% sodium dodecyl sulfate, phenol, and chloroform by the method of Marmur (13); the DNA isolation (13) included treatment of the DNA with Pronase at 100 μg/ml and RNase A at 20 μg/ml (heated at 90 C for 10 min to inactivate potential DNase) prior to the use of the DNA in the transfection experiment. This DNA had an absorbancy at 260 nm to absorbancy at 280 nm ratio of 1.98. The supernatant from the uninduced Ki-BALB nonproducer cells yielded no detectable foci or replicating virus as assayed by supernatant polymerase when assayed on the mink fibroblast cell line (5). For the transfection of the mink fibroblast cells the method of Graham and Van Der Eb (4) was used with slight modifications. Mink fibroblast cells (CCL64, American Type Culture Collection) were seeded at 10^5 cells per plate in 60-mm petri dishes in Dulbecco's modification of Vogt media containing 10% fetal calf serum (Colorado Serum Co.). The next day the media was removed, and the plates were washed twice with Dulbecco's modification of Eagle salts without fetal calf serum. The DNA was sheared 5× in a 1.0-ml plastic pipette as suggested by Levy et al. (9) prior to use. The DNA (30 μg) was then added to 0.5 ml of buffer containing 0.05 M *N*-2-hydroxyethyl-piperazine-*N'*-ethanesulfonic acid (pH 7.05), 0.05 M potassium chloride, 0.14 M sodium chloride, 0.25 g of $Na_2HPO_4 \cdot 7(H_2O)$ per liter, and 0.1% (wt/vol) dextrose. Calcium chloride was then added to the tube to a final concentration of 0.125 M, and the fine precipitate which occurred was allowed to form over a period of 20 min at room temperature. The 0.5 ml was poured onto the surface of the 60-mm plate and allowed to incubate on the cells for 30 min at room temperature. After 30 min, 3.0 ml of Dulbecco media containing 10% fetal calf serum was then added, and the plates were incubated for 18 h at 37 C. The solution was then removed, and the plates were rinsed once with media containing fetal calf serum, refed with 4.0 ml of media, and allowed to incubate at 37 C. After approximately 7 days, the cells from each 60-mm plate were transferred to a 100-mm plate; after another week of growth, approximately 10% of the cells were subcultured to a new 100-mm plate. The cells were then subcultured at this 1:10 ratio approximately each week. The supernatants were processed and assayed for viral reverse transcriptase in the presence of the synthetic polymer, poly(rA)·oligo(dT) (12-18) by methods fully detailed in earlier publications (15). The specific activity of the tritiated TTP used in the experiments was 30,000 counts/min per pmol. The [^{3}H]TMP incorporated represents a 0.01-ml aliquot of a 100-fold concentrate of supernatant fluid.

[b] Uninfected.

the supernatant of the mink fibroblast cell line. One week after transfection, no detectable polymerase was found in the supernatant of any of the transfected mink fibroblasts. However, as early as 13 days after transfection, a low level of viral polymerase was detected in two cultures; viral reverse transcriptase activity then increased about 50-fold in the supernatant from the positive mink fibroblast cells. In other cultures supernatant polymerase was detectable between 18 to 25 days after the initial transfection. Control studies showed that over 80% of the polymerase could be inhibited by antibody to type C murine viral polymerase, and the transfected, but not control, mink cells had levels of murine type C p30 antigen.

Since the DNA used to transfect the mink fibroblast also contained the Kirsten sarcoma virus proviral sequences, it was of interest to examine the plates for foci of the Kirsten sarcoma virus (Table 1, experiment 2). In repeated experiments, foci could not be visualized prior to detection of supernatant viral polymerase. Thus, approximately 1 month after transfection, when polymerase values were high (Table 1, experiment 1), the plates were examined for Kirsten sarcoma foci. On only 4 of the 11 plates which also had the replicating mouse type C virus(es) could the Kirsten sarcoma-transformed foci be detected. Neither foci nor polymerase was detected on each of four control plates treated with calcium phosphate but no DNA or from eight cultures treated with DNA from NIH 3T3 cells. By sedimentation in alkaline sucrose gradients, the DNA which was used for the transfection formed a broad peak from 10*S* to 40*S* in size distribution. In other experiments, treatment of the transfection solution with 5 μg of DNase I per ml but not 5 μg of RNase A per ml abolished the infectivity of the DNA. The results indicate that replicating murine type C virus(es) can be obtained by transfection of the mink cell with Ki-BALB

TABLE 2. *Host range of transfected virus*[a]

Virus	[^{3}H]TMP incorporated (pmol)						
	BALB/c[b]	NIH Swiss	Mink	SIRC	Duck	NRK	SC-1
Transfected virus	<0.03	<0.03	18.2	8.3	8.1	4.3	0.3
Xenotropic [S16 cl 10(I)]	<0.03	<0.03	13.1	14.2	11.3	5.2	0.4
N-tropic (Ki-MuLV)	0.5	22.3	<0.03	<0.03	<0.03	2.2	25.6
B-tropic (G-MuLV)	13.2	0.2	<0.03	<0.03	<0.03	1.1	19.5

[a] The host range of the replicating viruses isolated from experiment 2, culture 2, was tested in the following manner. The cells indicated were seeded in 60-mm plastic petri dishes at 10^5 cells per plate in Dulbecco media containing 10% calf serum and 2 μg of polybrene (Aldrich Chemical Co.) per ml. The next day viruses were filtered through 0.45-nm membrane filters (Millipore) and inoculated onto the indicated cells in 0.5 ml of media containing 2 μg of polybrene per ml by methods previously described (15). After a week of growth, the cells were transferred to 100-mm petri dishes (Falcon). After approximately 3 to 4 more days of growth, a 24-h harvest of supernatant fluid was tested for the presence of viral reverse transcriptase. The negative cultures were subcultivated another time and tested again 1 week later. The picomoles of TMP incorporated are indicated in a 0.01-ml aliquot obtained from a 100-fold concentrate of 10 ml of supernatant fluid from each of the indicated cultures in a 60-min 37 C reverse transcriptase reaction (15). The UV XC plaque test (13) was performed on parallel 60-mm plates 7 days after the initial infection without subculturing the original cells. The B-tropic strain of Gross leukemia virus and (G-MuLV) and N-tropic strain of Kirsten leukemia virus (Ki-MuLV) were grown on SC-1 cells as previously noted (15). The xenotropic virus, S16 C1 10(I), was the gift of George Todaro, National Cancer Institute (16). The source of the BALB 3T3, Ki-BALB, NIH 3T3, and NRK cells has been detailed earlier (15). The mink cell (CCL64), and SIRC cell (CCL60) used by Benveniste et al. and Henderson et al. (2, 5) and the Pekin duck cell (CCL 141) were from the American Type Culture Collection. The SC-1 cell was from Janet Hartley, National Institute of Allergy and Infectious Diseases.

[b] Cells tested.

DNA in the absence of transfection by the Kirsten sarcoma viral genome.

The host range of the replicating type C virus(es) isolated in the mink cell was examined by testing the virus for growth on a variety of mouse cells and heterologous cells (Table 2). The replication of the virus was followed by the viral supernatant polymerase, and in addition on the mouse cells by the XC plaque assay (14). The virus obtained by transfecting the mink cell grew readily in a rabbit cell line (SIRC), in the mink cell, in a rat fibroblast (NRK), and a duck cell; it did not grow to detectable levels in BALB 3T3 or NIH 3T3 cells and did not form XC plaques in BALB 3T3, NIH 3T3, or SC-1 cells. Positive controls showed that an N-tropic virus grew and formed XC plaques in NIH 3T3 and SC-1 cells, and that a B-tropic virus grew and formed XC plaques in BALB 3T3 cells or SC-1 cells. Low levels of replication by the transfected virus(es) were detected in the SC-1 cell line as assayed by viral supernatant polymerase. By comparison, the host range of the transfected virus(es) resembled another xenotropic virus, S16 cl 10(I) (16). The results indicate that the virus(es) isolated from the Ki-BALB DNA has the ability to replicate in a variety of heterologous cells but not in mouse cells, and thus can be denoted as a xenotropic mouse type C virus(es). A full characterization of the transfected virus(es) and detailed comparisons to previous isolates of xenotropic viruses will be the subject of a later report.

The current experiments indicate that an endogenous xenotropic type C virus can be isolated from Ki-BALB cells by isolating the DNA from that cell and transfecting a permissive cell with that DNA preparation. The generality of the transfection method for the isolation of other endogenous viruses from their natural hosts is not known. Uninduced Ki-BALB cells are known to release xenotropic virus at a low level, approximately 1 cell in 10^6 cells by infectious center assays (1). We cannot exclude that some level of virus expression by the Ki-BALB cell is necessary for the transfection with the isolated DNA to be successful. The BALB/c endogenous xenotropic virus(es) cannot reinfect exogenously the cells in which they normally reside. Thus, if "leakiness" is necessary, the mechanism by which such leakiness of virus would allow the naked DNA to be transfectable to heterologous cells will be important to understand.

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ADDENDUM IN PROOF

An RD-114 type virus can be isolated from a continuous line of cat cells, CCC, by transfection of a dog thymus cell with DNA from CCC cells not releasing virus.

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