Proc. Nat. Acad. Sci. USA
Vol. 71, No. 3, pp. 602–606, March 1974

A Distinct Class of Inducible Murine Type-C Viruses that Replicates in the Rabbit SIRC Cell Line

(RNA tumor viruses/endogenous virogene expression/RNA•DNA hybridization)

RAOUL E. BENVENISTE, MICHAEL M. LIEBER, AND GEORGE J. TODARO

Viral Leukemia and Lymphoma Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

Communicated by Henry S. Kaplan, August 31, 1973.

ABSTRACT **The existence of the selectively permissive rabbit cell line SIRC allows definition of a new class of endogenous murine type-C virus. Continuous clonal lines of transformed cells derived from the BALB/c mouse-embryo cell line BALB/3T3 contain at least two distinct classes of endogenous type-C viral genomes. Spontaneously released endogenous viruses grow well on the mouse cell line NIH/3T3 (N-tropic viruses) but not on the rabbit cell line SIRC. Type-C viruses induced by treatment with BrdU grow well on SIRC (S-tropic viruses) but not in NIH/3T3 or BALB/3T3. BrdU-treated AKR mouse-embryo cells also release an S-tropic virus. N-tropic and S-tropic viruses both have the mouse intraspecies gs-1 and viral RNA-directed DNA polymerase antigenic determinants. DNA·RNA hybridization techniques reveal that the two host-range classes of endogenous viruses are only partially related to each other. Cell transformation facilitates the spontaneous release of the N-tropic viruses; treatment with thymidine analogues induces the production of the S-tropic viruses. Thus, the two classes of viral genomes appear to be subject to different cellular controls.**

AKR and BALB/c mouse-embryo cells contain endogenous type-C viral genomes that can be induced to full viral expression by treatment with 5-bromodeoxyuridine (BrdU) and 5-iododeoxyuridine (IdU) (1, 2). The availability of transformed derivatives of the BALB/3T3 cell line (3, 4) that are "super-inducible" for endogenous type-C viral expression (5) prompted a study of the kinetics of the induction of endogenous type-C viral specific RNA after BrdU treatment. However, it proved impossible to demonstrate that the cytoplasmic RNA extracted from BALB/3T3 derived super-inducible cell lines after treatment with BrdU contained sequences fully homologous to DNA probes made from spontaneously released endogenous type-C viruses. This unexpected result suggested that BALB/c derived cell lines might contain more than one type of endogenous type-C viral genome, and stimulated a search for a cell line that would be permissive for replication of the "induced" endogenous type-C virus. Aaronson and Stephenson have described the independent segregation of two genetic loci for induction of endogeneous type-C viruses from BALB/c mouse-embryo cells (6). One locus codes for activation of a virus that grows in NIH Swiss cells; the second codes for activation of a virus that can replicate in the rat NRK cell line.

Studies of the RD/CCC class of endogenous feline viruses had shown that various primate cells, as well as bat, horse, buffalo, and mink cells, permit viral replication (7). Thus, it could not be predicted *a priori* what characteristics a cell line need have to be a permissive host for a given endogeneous type-C virus. Among many mammalian cell lines tested, only the rabbit cell line SIRC readily allows replication of an induced virus from BALB/3T3 and its derivatives. SIRC-tropic viruses have also been induced by BrdU treatment of AKR embryo cell lines. Thus, the SIRC cell line permits the isolation and characterization of a new class of murine endogenous type-C viruses.

Abbreviation: gs-1 antigen, group-specific antigen.

MATERIALS AND METHODS

Cells. Cell lines were maintained in plastic tissue culture flasks or in glass roller bottles with Dulbecco's modification of Eagle's medium supplemented with 10% calf serum. The origins of the host cell lines used to test for type-C viral replication were: DBS-FRhL-1 (rhesus monkey), generously supplied by Dr. Rosyln Wallace (8); A101 (human malignant melanoma), simian virus (SV) clone 80 (SV40-transformed human fibroblasts), and NIH/3T3 (NIH Swiss mouse) (9), derived in this laboratory; FFc60WF (fetal-cat cell strain), Naval Biological Research Laboratory; and RK 13 (rabbit), Flow Laboratories. The remaining host cell lines were obtained from the American Type Culture Collection. The BALB/3T3 methylcholanthrene-induced transformant MC5-5 was generously supplied by Dr. Y. Ikawa.

RNA-Directed DNA Polymerase Assay. Putative virus in tissue culture medium was concentrated by centrifugation. RNA-directed DNA polymerase activity was assayed with synthetic template and primer as described (10). Results are expressed as counts per minute of [^{3}H]TMP incorporated into radioactive poly(dT) product in a 60-min incubation at 37°. Polymerase antibody inhibition studies were performed as described (11).

Group-Specific Antigen Assay. A radioimmunoprecipitation assay for the murine intraspecies group-specific (gs-1) antigen was performed as described (12). Cells were extracted as described (4).

DNA·RNA Hybridization. Supernatant type-C virus was purified by isopycnic banding on sucrose gradients. 70S viral RNA was extracted from purified virions by disruption with 1.0% Na dodecyl sulfate, followed by velocity sedimentation in a sucrose density gradient (13). Cytoplasmic RNA was extracted from cells (14). The endogenous RNA-directed DNA polymerase reaction from detergent-disrupted type-C virus was used to synthesize [^{3}H]thymidine-labeled DNA in the presence of 50 μg/ml of actinomycin D (14, 15). About 2000

cpm (0.1 ng) of enzymatically synthesized [^{3}H]DNA was incubated with 10^2- to 10^7-fold excess of either viral or cytoplasmic RNA for 72 hr at 41° in 0.20-ml reaction mixtures containing 15 mM Tris·HCl, pH 7.5; 0.15 M NaCl; 0.5 mM EDTA; 0.1% Na dodecyl sulfate; and 38% formamide (15). RNA·DNA hybrids were detected by hydrolysis with S1, a single-strand specific nuclease as described (14).

Virus Induction and Infectivity Tests. BrdU (Calbiochem Corp.) was used to induce type-C virus production. Cell lines were treated for 24 hr with 100 μg/ml of BrdU dissolved in cell culture medium. The infectivity assays were performed by transmitting 1.5 ml of supernatant fluid, filtered through 0.45-μmMillipore filters, directly onto host cell lines (10^6 cells per tissue culture flask) that had been treated for 24 hr with complete medium containing 2 μg/ml of polybrene.

RESULTS

Fig. 1 is a genealogy of the BALB/c mouse-embryo derived cell lines used. BALB/3T3 (clone A31) is a contact-inhibited cell line that does not spontaneously release endogenous type-C virus (3, 4). R4, a tumorigenic survivor of 1500 rads of x-radiation, has a transformed morphology and does not spontaneously release endogenous type-C virus in culture (16). S2CL-3 is a spontaneous epithelioid transformant of BALB/3T3 that began spontaneously to release high titers of endogenous type-C virus (3). S16 is a fibroblastic spontaneous transformant that does not release virus. S16CL-10 is a virus nonproducing subclone of S16; S16CL-2 is another subclone that spontaneously releases endogenous virus (4).

Host Range Was Determined by passing filtrates of tissue culture medium onto virus nonproducing host cell lines of several types. This method causes major losses of infectious virus particles. Therefore, this technique could lower the titer of a given virus below that necessary for infectivity in certain cell lines, and, thereby, exaggerate apparent host-range restrictions. However, the filtration method clearly measures the extracellular production of complete, fully infectious virus from the cell line under study. Cocultivation, while more sensitive for detection of low levels of infectious virus, might also allow the "rescue" of incomplete virus by permissive host cells.

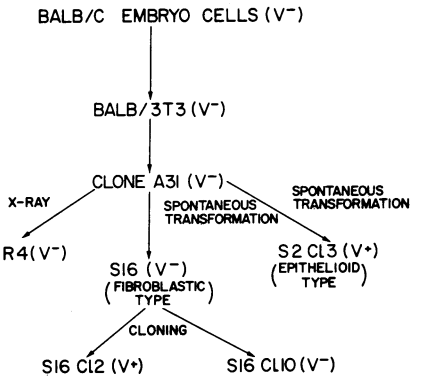

FIG. 1. Genealogy of BALB/3T3 derived cell lines. V^+ indicates cell lines that spontaneously release endogenous type-C virus; V^- cell lines do not spontaneously release virus. All these cell lines, including secondary BALB/c embryo cells, contain endogenous type-C viruses inducible by treatment with halogenated pyrimidines (2, 3, 24).

Subsequent replication of type-C virus was quantitated by assaying the supernatant viral RNA-directed DNA polymerase activity of the host cell cultures. With the assay used, mouse cell lines not releasing type-C virus show less than 2000 cpm (0.05 pmol) of [^{3}H]TMP incorporated. The endogenous viruses released spontaneously from S16CL-2 and S2CL-3 replicate well in NIH/3T3 cells, but not in BALB/3T3 derived cells nor in the rabbit cell line SIRC (Table 1). The BALB/3T3 cell line and its transformed progeny, R4 and S16CL-10, do not spontaneously release type-C viruses that can replicate in any of the host cell lines listed. However, if these cell lines are treated with BrdU (100 μg/ml for 24 hr),

TABLE 1. *Host range of spontaneously released and BrdU-induced BALB/3T3 derived endogenous viruses**

Host cells		Untreated control flask	Source of virus								
Species	Cell line		S16CL-2	S2CL-3	BALB/3T3I†	R4I	S16CL-10I	S16CL-2I	S2CL-3I	R4/B	Rauscher MuLV
			(cpm × 10^{-3} of [^{3}H]TMP incorporated)								
Mouse (BALB/c)	BALB/3T3 clone A31	0.5	1.7	2.0	1.9	1.5	0.8	1.2	1.5	*85.0*	*1657.5*
Mouse (BALB/c)	R4	0.7	0.9	1.1	0.6	0.8	1.3	0.9	2.4	*952.6*	*369.3*
Mouse (BALB/c)	S16CL-10	0.8	1.7	1.3	NT‡	1.3	0.7	1.7	2.8	*411.6*	*595.0*
Mouse (NIH/Swiss)	NIH/3T3	0.9	*304.2*	*181.2*	0.9	0.5	0.9	*388.1*	*146.1*	1.1	*1237.4*
Rabbit	SIRC	1.2	2.1	1.9	*115.3*	*370.1*	*183.7*	*312.7*	*44.0*	1.1	2.6
Rat	NRK	2.5	4.5	2.0	2.8	2.1	3.5	5.1	3.6	*32.5*	*409.7*

* Medium from the designated virus-producing cultures was filtered and used to infect the host cell lines listed. Virus replication in these host cell lines was ascertained by assaying their tissue culture media for RNA-directed DNA polymerase activity. Culture media were tested 14–18 days after infection. The results listed represent the highest values obtained from two or more separate experiments; values have been multiplied by 10^{-3}. *Italicized values* represent more than 20 × 10^3 cpm.

† "I" indicates that these cell lines were induced with BrdU. Infection experiments were performed 3 or 4 days later at the peak of virus secretion (4).

‡ *NT* = not tested.

TABLE 2. *Murine gs-1 antigen assay*

Cell line	Highest positive titer
SIRC	<1:2
BALB/3T3 (clone A31)	1:2
S16CL-10	1:2
BALB/3T3 (clone A31) I*	1:64
S16CL-10I*	1:16
S16CL-2	1:256
SIRC infected with S16CL-10I	1:128
SIRC infected with S16CL-2I	1:512
SIRC infected with S2CL-3I	1:256

* These cell lines were treated with BrdU (100 μg/ml) for 24 hr beginning 72 hr before they were collected.

infectious type-C viruses are induced. The viruses induced from BALB/3T3, R4, and S16CL-10 replicate well in the rabbit SIRC cell line, but do not replicate to a detectable extent in NIH/3T3, rat NRK cells, or in any of the BALB/c derived cell lines (Table 1). Moreover, if the spontaneous producer cell lines S16CL-2 and S2CL-3 are treated with BrdU, they also release a virus that replicates well in the SIRC cell line. Both the BALB-(B)-tropic virus released by the R4/B cell line, and Rauscher murine leukemia virus, which is both N-(NIH Swiss) and B-tropic (17), are unable to replicate in SIRC (Table 1).

Antibody inhibition studies were performed in order to compare the polymerases from the induced and spontaneously released viruses. Serum prepared against purified Rauscher viral polymerase exhibits no significant differences in its ability to inhibit the polymerases of Rauscher virus, S2CL-3 spontaneously released virus, or S16CL-10 induced (S16CL-10I) virus growing on the SIRC cell line (data not shown).

A radioimmunoassay for the murine intraspecies gs-1 antigenic determinant confirms the murine origin of the virus growing on SIRC. The SIRC cell line itself contains no measurable murine gs-1 activity (Table 2). A small amount of gs-1 activity is normally observed in the nonproducer mouse cells

TABLE 4. *Induction of type-C viruses from mouse cells other than BALB/c that replicate in the SIRC cell line*

Cell lines tested*	SIRC cultures 16 days after infection
	cpm × 10^{-3} of [^{3}H]TMP incorporated
Uninfected SIRC control culture	0.9
AKR (secondary embryo cell strain)	1.2
AKR I	*898.6*
NIH/3T3	0.6
NIH/3T3 I	0.6
3T3	1.0
3T3 I	1.2
S16CL-10	0.8
S16CL-10I	*357.1*

* "I" indicates cell lines that were treated with BrdU, 100 μg/ml for 24 hr. Media from treated and untreated cultures were used to infect SIRC cultures 3 days after BrdU treatment commenced. Viral replication in SIRC was measured by the supernatant RNA-directed DNA polymerase assay. *Italicized values* represent more than 20 × 10^3 cpm.

(18); an increased concentration of antigen is detected after treatment with BrdU (as shown for BALB/3T3 and S16CL-10). The SIRC cell line growing the induced endogenous BALB/c viruses S16CL-10I, S16CL-2I, and S2CL-3I show levels of murine gs-1 comparable to that of S16CL-2, a typical spontaneous producer of endogenous virus. These studies show that the gs-1 antigenic determinants of the induced and spontaneously released endogenous viruses both react with antisera prepared against purified Rauscher virus antigen.

Host cell lines from other mammalian species were tested to identify additional host-range differences between the spontaneously released and the induced endogenous BALB/3T3 viruses. The induced virus from S16CL-10 is unable to replicate in a wide variety of mammalian cell lines (Table 3). No other cell lines derived from rabbit tissue other than the SIRC line support replication of S16CL-10I. However, in-

TABLE 3. *Host range of the endogenous viruses**

Species	Cell line	Uninfected control	S16CL-2	S16CL-10I†	S16CL-10I grown in SIRC
		(cpm × 10^{-3} of [^{3}H]TMP incorporated)			
Human	A101	1.1	1.3	1.2	1.2
Human	SV80	2.0	2.5	2.3	1.6
Rhesus monkey	DBS-FRhL-1	4.0	4.7	3.7	*24.5*
Cat	FFc60WF	2.5	2.8	2.6	*125.3*
Buffalo	Bu (IMR-31)	0.7	0.6	0.6	*295.9*
Rabbit	SIRC	0.9	0.8	*409.2*	*1137.6*
Rabbit	SflEp (NBL-11)	2.2	3.0	1.2	0.9
Rabbit	TRK-1	1.4	1.6	1.7	*497.6*
Rabbit	RK-13	4.6	6.1	8.2	*56.7*
Rat	NRK	2.0	2.5	3.5	*112.8*

* Host range for replication of the spontaneously released S16CL-2 and induced S16CL-10I viruses, as well as of the S16CL-10I virus growing in SIRC, was ascertained by passing filtered culture media of these cultures onto the host cell lines listed. Virus replication was subsequently measured by an assay for viral RNA-directed DNA polymerase activity 14–18 days after infection. Virus titers by the polymerase assay of the concentrated supernatants used for these infections: S16CL-2, 500 × 10^3 cpm; S16CL-10I, 15 × 10^3 cpm; S16CL-10I grown in SIRC, 500 × 10^3 cpm. *Italicized values* represent more than 20 × 10^3 cpm.

† "I" indicates that these cell lines were treated with BrdU. Infection experiments were performed 3 or 4 days later at the peak of virus secretion (4).

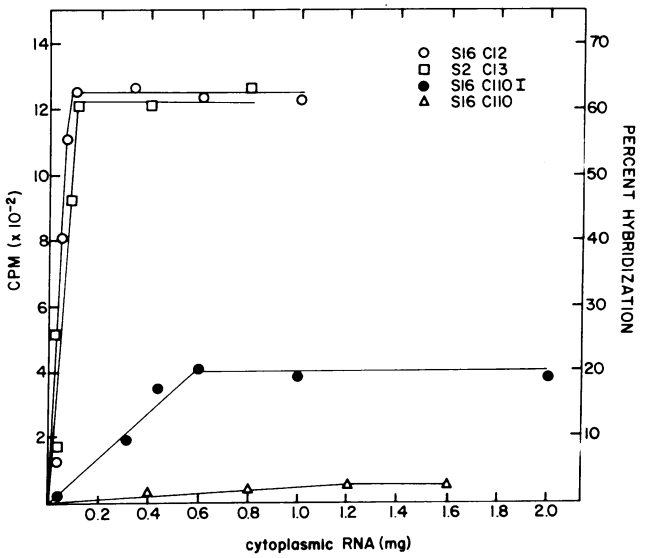

FIG. 2. Hybridization of S16CL-2 [^{3}H]DNA (2000 cpm) product to cytoplasmic RNA extracted from cultures releasing type-C virus either spontaneously or after induction with BrdU. (O) cytoplasmic RNA extracted from S16CL-2 cells; (□) RNA from S2CL-3 cells; (●) RNA extracted from S16CL-10 cells 72 hr after induction with BrdU; (△) RNA from S16CL-10 uninduced cells. The radioactivity shown represents [^{3}H]DNA product resistant to digestion by nuclease S_1. Background levels [1–3% of the input radioactivity (14)] have been subtracted. Values have been multiplied by 10^{-2}.

creasing the titer of the S-tropic virus by passage through SIRC cells greatly expands the host range for replication. The SIRC-grown virus replicates on rhesus monkey, cat, buffalo, rat, and two other rabbit cell lines. Inhibition studies with anti-mouse polymerase are consistent with a murine origin for the viruses growing in cells of these various species. S16CL-2 virus is unable to replicate in any of these cell lines.

Experiments to determine if cell lines from other mouse strains also contain inducible viruses that replicate well on the SIRC cell line are shown in Table 4. A secondary AKR mouse-embryo culture releases a virus with this property after treatment with BrdU, indicating that this mouse strain also has an S-tropic virus. It has not been possible so far to induce this virus class from NIH/3T3, nor from the random bred mouse line 3T3 (19).

Nucleic Acid Homology Studies. The relatedness of type-C viruses can be examined by comparing their nucleic acid homology by DNA·RNA hybridization. Single-stranded [^{3}H]DNA copies of S16CL-2 and S2CL-3 viral RNAs prepared by using the endogenous RNA-dependent DNA polymerase in the presence of actinomycin D were annealed to cytoplasmic RNA extracted from various cell lines. Fig. 2 shows representative data obtained after digestion of the RNA·DNA hybrids with the single-strand specific nuclease, S1 (14). The spontaneous producer cell lines S2CL-3 and S16CL-2 contain RNA that hybridizes to the [^{3}H]DNA product made from S16CL-2 virus; saturating levels are reached after the addition of 30 μg of cytoplasmic RNA, and represent 62% of the input [^{3}H]DNA radioactivity. A similar level of hybridization is also obtained with 0.5 μg of 70S RNA extracted from these viruses (data not shown). Cytoplasmic RNA extracted from S16CL-10 cells 72 hr after treatment with 100 μg/ml of BrdU (at the peak of virus induction) hybridizes to only 20% of the S16CL-2 [^{3}H]DNA probe. This level of hybridization is also found with RNA extracted from the virus produced by the induced S16CL-10 culture. Comparable saturation levels are obtained with [^{3}H]DNA product made from S2CL-3 virus. The virus induced from S16CL-10 cells is therefore only partially homologous to the spontaneously produced viruses.

RNA extracted from uninduced S16CL-10 cells saturates about 3% of the DNA probe. This low level of hybridization has been shown for various BALB/c derived nonproducer cell lines, and seems to represent a partial transcription of type-C virus specific information (14). Whether this information is due to the "N-tropic", "S-tropic", or still other endogenous viral genomes has not been resolved.

The cytoplasmic RNAs extracted from other transformed nonproducer BALB/3T3 derived cell lines [such as MC5-5, S16CL-1, and S16CL-11 (4)] after treatment with BrdU also saturate 20% of the [^{3}H]DNA products prepared from either S16CL-2 or S2CL-3 viruses. The simultaneous addition of saturating concentrations of RNA extracted from each of these induced cell lines does not result in an additional extent of hybridization to the S16CL-2 DNA product. The viruses induced from these various cell lines therefore share common sequences with each other and with the spontaneously released viruses. However, the viruses induced from these cell lines, like S16CL-10I, are also only partially homologous to the spontaneously released viruses.

DISCUSSION

The rabbit cell line SIRC was derived from normal rabbit cornea cells by M. Volbert of the Staatens Seruminstitut, Copenhagen. It is useful for the primary isolation of rubella virus (20, 21), but has not previously been used for the isolation of mammalian type-C viruses. BALB/3T3 and transformed cell lines derived from it contain at least two distinct classes of endogenous type-C viral genomes. Certain spontaneously released viruses replicate well in NIH/3T3 cells (N-tropic viruses) but do not replicate in the rabbit SIRC cell line. After treatment with BrdU, both spontaneous producer and nonproducer BALB/3T3 derived cell lines yield type-C viruses that replicate readily in the SIRC cell line ("S-tropic viruses"). Thus, separate control mechanisms appear to govern full expression of these two classes of endogenous type-C virogenes in BALB/3T3 and its derived cell lines.

Filtered supernatant fluids from induced nonproducer cultures do not contain detectable levels of virus able to replicate in NIH/3T3. Studies have demonstrated the induction of N-tropic viruses from BALB/c cells, but only after cultivation of the treated cells with NIH/3T3, and not by infection with filtered supernatants (1, 23). Hybridization studies also show that less than 0.5% of the total viral RNA induced after BrdU treatment is homologous to the N-tropic virus probe. The N-tropic virus is probably induced in such low titer that it can only be detected by the more sensitive cocultivation assay.

DNA·RNA hybridization studies indicate that the N-tropic and S-tropic viruses have different nucleic acid sequences in their RNA genomes. There is only a partial homology (30–35%) between DNA probes made from the N-tropic spontaneously released viruses and RNA extracted from maximally induced S-tropic virus releasing lines. The spon-

taneously released N-tropic murine virus (S2CL-3) shows more homology (22) with Kirsten murine leukemia virus (90–100%), Rauscher murine leukemia virus (57%), and, even, AT-124 virus (48%), which was isolated from human tumor cells growing in immunosuppressed NIH Swiss mice (23). The S2CL-3 DNA probe shows almost as much homology with the RNA from two presumed primate type-C viruses [woolly monkey (25%) and gibbon ape (24%)] as it does with the induced mouse S-tropic virus RNA (22).

The "BALB virus-2" described recently was isolated by cultivation of induced BALB/c derived cells with rat NRK cell line (6). We could not isolate inducible virus on NRK using filtered supernatants. "BALB virus-2" and the S-tropic viruses described here are probably representatives of the same inducible virus class, but the SIRC cell appears to be a more sensitive indicator cell for this class of viruses. In addition, NRK cells are less useful for isolation of this class of viruses because of the high probability of activation of an NRK endogenous virus (10) and because "laboratory" strains such as Rauscher leukemia virus replicate readily in NRK but not SIRC (see Table 1).

There have been reports of the isolation of B-tropic type-C viruses from aged BALB/c animals (25). Type-C viruses with a B-tropic host range have not been induced in our laboratory by "short-term" BrdU treatment from R4 or other BALB/3T3 derived cell lines. However, the R4/B cell line derived in Dr. G. Barski's laboratory (26) by chronic treatment of the nonproducer R4 cell line with BrdU in order to select for cells that lacked thymidine kinase releases high titers of a BALB-tropic type-C virus (see Table 1). If this virus is also an endogenous virus, it represents yet another host-range class present in the original BALB/3T3 genome (27).

The endogeneous type-C viruses of AKR mouse derived cells lines have been studied by Rowe and his coworkers. These cell lines can spontaneously release N-tropic viruses (27); N-tropic viruses can also be induced by IdU treatment (1). The experiment reported here demonstrates that AKR embryo cells also contain an inducible "S-tropic" virus. Thus, cells from AKR mice contain different classes of endogenous type-C viral genomes with host-range properties similar to those found in BALB/c derived cells.

We thank Isabella Musto, Linda Papke, Debbie Searfoss, Janice Van Knapp, and Gaye Lynn Wilson for their help. This research was supported in part by the Cancer Virus Program of the National Institutes of Health.

1. Lowy, D. R., Rowe, W. P., Teich, N. & Hartley, J. W. (1971) *Science* **174**, 155–156.
2. Aaronson, S. A., Todaro, G. J. & Scolnick, E. M. (1971) *Science* **174**, 157–159.
3. Todaro, G. J. (1972) *Nature* **240**, 157–160.
4. Lieber, M. M. & Todaro, G. J. (1973) *Int. J. Cancer* **11**, 616–627.
5. Lieber, M. M., Livingston, D. M. & Todaro, G. J. (1973) *Science* **181**, 443–444.
6. Aaronson, S. A. & Stephenson, J. R. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2055–2058.
7. Todaro, G. J., Benveniste, R. E., Lieber, M. M. & Livingston, D. M. (1973) *Virology* **55**, 506–515.
8. Wallace, R. E., Vasington, P. J., Petricciani, J. E., Hopps, H. E., Lorenz, D. E. & Kadanka, Z. (1973) *In Vitro* **8**, 333–341.
9. Jainchill, J. L., Aaronson, S. A. & Todaro, G. J. (1969) *J. Virol.* **4**, 549–553.
10. Lieber, M. M., Benveniste, R. E., Livingston, D. M. & Todaro, G. J. (1973) *Science* **182**, 56–59.
11. Parks, W. P., Scolnick, E. M., Ross, J., Todaro, G. J. & Aaronson, S. A. (1972) *J. Virol.* **9**, 110–115.
12. Parks, W. P., Scolnick, E. M. & Livingston, D. M. (1972) *J. Immunol.* **109**, 570–577.
13. Ross, J., Tronick, S. & Scolnick, E. M. (1972) *Virology* **49**, 230–235.
14. Benveniste, R. E., Todaro, G. J., Scolnick, E. M. & Parks, W. P. (1973) *J. Virol.* **12**, 711–720.
15. Benveniste, R. E. & Scolnick, E. M. (1973) *Virology* **51**, 370–383.
16. Pollock, E. J., Aaronson, S. A. & Todaro, G. J. (1970) *Int. J. Radiat. Biol.* **17**, 97–100.
17. Hartley, J. W., Rowe, W. P. & Huebner, R. J. (1970) *J. Virol.* **5**, 221–225.
18. Parks, W. P., Livingston, D. M., Todaro, G. J., Benveniste, R. E. & Scolnick, E. M. (1973) *J. Exp. Med.* **137**, 622–635.
19. Todaro, G. J. & Green, H. (1963) *J. Cell Biol.* **17**, 299–313.
20. Leerhoy, J. (1965) *Science* **149**, 633–634.
21. Phillips, C.A., Melnick, J. L. & Burkhardt, M. (1966) *Proc. Soc. Exp. Med. Biol.* **122**, 783–786.
22. Benveniste, R. E. & Todaro, G. J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3316–3320.
23. Todaro, G. J., Arnstein, P., Parks, W. P., Lennette, E. H. & Huebner, R. J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 859–862.
24. Stephenson, J. R. & Aaronson, S. A. (1972) *J. Exp. Med.* **136**, 175–184.
25. Peters, R. L., Hartley, J. W., Spahn, G. J., Rabstein, L. S., Whitmire, C. E., Turner, H. C. & Huebner, R. J. (1972) *Int. J. Cancer* **10**, 283–289.
26. Barski, G., Blanchard, M. G., Youn, Y. K. & Leon, B. (1973) *J. Nat. Cancer Inst.* **51**, 781–792.
27. Rowe, W. P., Hartley, J. W., Lander, M. R., Pugh, W. E. & Teich, N. (1971) *Virology* **46**, 866–876.