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Homology Between Type-C Viruses of Various Species as Determined by Molecular Hybridization

(RD-114 virus/primate viruses/endogenous viruses/RNA·DNA hybridization/S_1 nuclease)

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ABSTRACT **Two strains of feline leukemia virus, two endogenous feline type-C viruses (RD/CCC group), several endogenous and laboratory strains of murine "leukemia" virus, two rat viruses, two primate viruses (woolly monkey and gibbon ape), as well as hamster, pig, and avian type-C viruses were examined for their relatedness to one another by molecular hybridization. The extent of nucleic-acid homology was determined by hybridization of the various viral RNAs to a [^{3}H]DNA product synthesized from each virus. Among the murine type-C viruses (Rauscher, Kirsten, AT-124, and endogenous BALB/c virus) a high degree of homology is observed, although the viruses are not identical. The two primate viruses are also closely related to one another. The feline, rat, hamster, and pig endogenous viruses can be readily distinguished from one another and from the murine and primate viruses since their DNA products share very little or no nucleic-acid homology. However, the murine and primate type-C virus groups possess a surprising degree of relatedness. Feline type-C viruses fall into two distinct groups, the feline leukemia virus group and the RD-114/CCC group, with little detectable nucleic-acid homology between them. Infection of feline or rat cells with type-C virus results in production of the endogenous type-C virus of the species along with the infecting virus.**

Reptilian, avian, murine, rat, hamster, porcine, feline, and primate type-C RNA viruses have been isolated (1, 2). Some of these viruses are produced spontaneously (3, 4), others after induction of virus-free cell clones by chemicals such as 5'-bromodeoxyuridine (5, 6). Radioimmunoassays for the species-specific antigen of the viral group-specific protein (7, 8) and inhibition of the viral RNA-dependent DNA polymerases with antisera prepared against various type-C virus polymerases (9, 10) have demonstrated immunological differences in these two proteins among the various type-C virus isolates. Haapala and Fischinger (11) have reported on the nucleotide sequence diversity among feline, murine, and RD-114 type-C viruses as determined by RNA·DNA hybridization with hydroxylapatite chromatography. To extend these comparative studies, we have systematically examined the homologies between various type-C viruses by nucleic-acid hybridization.

MATERIALS AND METHODS

Cells were grown in Dulbecco's modification of Eagle's minimal essential medium containing 10% calf serum (Colorado Serum Co., Denver, Colo.) at 37° in a 10% CO_2 atmosphere. S2Cl3 is a morphologically transformed cell line derived from BALB/3T3 clone A31; it continuously produces high titers of endogenous mouse type-C virus (12). The normal rat-kidney cell line (NRK) has been described (13). Virus-transformed NRK cells include a nonproducer transformed by Kirsten sarcoma virus, K-NRK clone 32 (14), and a line transformed by Kirsten sarcoma virus which produces both the Kirsten strain of leukemia virus and sarcoma virus. SV-A clone 80 (SV clone 80) is a human diploid skin-fibroblast cell line transformed by Simian virus 40 (15). A204 is a cultured human rhabdomyosarcoma line developed in our laboratory. RD is a human rhabdomyosarcoma cell line originally described by McAllister *et al.* (16).

Viruses. Table 1 lists the viruses that we examined for nucleic-acid homology and the cell lines on which they were grown. Rauscher murine leukemia virus (R-MuLV), and the Gardner–Arnstein strain of feline leukemia virus were obtained from Electro-Nucleonics Laboratories, Silver Spring, Md. The Rickard strain of feline leukemia virus (R-FeLV) was obtained from University Laboratories (Highland Park, N.J.). RD-114 virus was supplied by Pfizer Laboratories, Maywood, N.J. Avian myeloblastosis virus (AMV) was kindly provided by Dr. Joseph Beard, Duke University. The remaining viruses were grown and purified in our laboratory. All virus preparations were banded twice in sucrose density gradients before being used.

Extraction of Viral and Cytoplasmic RNA. 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 (17). Cytoplasmic RNA was extracted from cells (18).

Synthesis and Purification of Viral [^{3}H]DNA. The endogenous RNA-dependent DNA polymerase reaction from type-C virus disrupted by detergent was used to synthesize [^{3}H]thymidine-labeled DNA in the presence of 50 μg/ml of actinomycin D (18, 19).

Hybridization and Analysis of RNA·DNA Hybrids. 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 7.4), 0.15 M NaCl, 0.5 mM EDTA, 0.1% Na dodecyl sulfate, and 38% formamide (19).

Abbreviations: NRK cells, normal rat kidney cells; R-MuLV and Ki-MuLV, Rauscher and Kirsten murine leukemia virus, respectively; R-FeLV, Rickard feline leukemia virus; AMV, avian myeloblastosis virus.

TABLE 1. *Type-C viruses examined for homology*

Family	Virus	Host cell line
Feline	FeLV (Rickard strain)	Cat thymus cells (F422)
	FeSV (Gardner–Arnstein strain)	Cat embryo cells
	CCC	CCC cat kidney cell (endogenous)
	RD-114	Human rhabdomyosarcoma (RD)
Murine	S2Cl3	Derivative of BALB/3T3 clone A31 (endogenous)
	Rauscher MuLV (R-MuLV)	Murine (JLSV-9)
	Kirsten MuLV (Ki-MuLV)	Murine (NIH/3T3)
	Ki-SV (MuLV)	Murine sarcoma virus (MSV)-transformed rat-kidney (NRK) cell line
	AT-124	Human rhabdomyosarcoma (RD)
Rat	V-NRK	NRK clone 3 (endogenous)
	CCL-38	Rat-carcinoma cell line* (LLC-WRC 256) (endogenous)
Hamster	CCL 14.1	Chinese hamster-peritoneal cell line* (B14-I50) (endogenous)
Pig	CCL-33	Pig kidney line (PK-15)*
Primate	Woolly (SSV-1)	Normal rat kidney (NRK)
	Woolly (SSV-1)	Human rhabdomyosarcoma (A204)
	Gibbon	Simian virus 40 (SV40)-transformed human skin fibroblasts (SV80)
Avian	AMV	Infected chicken plasma

* Obtained from the American Type Culture Collection, Rockville, Md.

RNA·DNA hybrids were detected by hydrolysis with S_1, a nuclease specific for single strands (18).

RESULTS

Table 1 lists the various type-C viruses that we examined for relatedness by hybridization. A single-stranded [^{3}H]DNA product synthesized in the presence of actinomycin D by the endogenous reaction of the RNA-directed DNA polymerase from the various virus preparations was annealed to 60–70S RNA extracted from purified virus or to cytoplasmic RNA extracted from cultures producing these viruses.

S2Cl3 cells and NRK cells producing Ki-SV(MuLV) contain RNA that hybridizes to the [^{3}H]DNA product prepared from Ki-MuLV. Saturating levels of hybridization were achieved with 60 μg of cytoplasmic RNA, and represented 68–70% of the total input [^{3}H]DNA radioactivity (Fig. 1*A*). RNA extracted from SV80 cells producing gibbon type-C virus hybridizes to 19% of the Ki-MuLV DNA probe; RNA extracted from the culture producing endogenous rat virus recognizes only about 5% of the nucleic-acid sequences, and RNA extracted from a cell line producing pig type-C virus or from SV80 cells not producing virus saturates less than 2% of the Ki-MuLV DNA probe. Fig. 1*B* shows the results obtained after hybridization of viral RNA to the [^{3}H]DNA product prepared from an endogenous BALB/c virus, S2Cl3. Viral RNA extracted from either S2Cl3 or from Ki-SV(MuLV) hybridizes to 66% of the probe, while RNA extracted from the Rauscher strain of MuLV only hybridizes to about 35% of the S2Cl3 DNA. Viral RNA extracted from woolly monkey virus grown in human cells is homologous to about 19% of the probe; in marked contrast, RNA extracted from AMV or RD-114 virus recognizes less than 1% of this mouse type-C virus probe.

Homology Between Type-C Viruses. Table 2 summarizes the data that we have obtained concerning the nucleic-acid sequence homologies of type-C viruses. Single-stranded [^{3}H]-DNA products prepared from various feline, murine, rat,

TABLE 2. *Homology between type-C viruses as determined by RNA·DNA hybridization*

(Percent hybridization*)

RNA† / [^{3}H]DNA‡	Murine				Rat		Feline				Primate		Hamster	Pig	Avian	Mason–Pfizer
	S2Cl3	R-MuLV	Ki-MuLV	AT-124	V-NRK	CCL-38	FeLV (Rickard)	FeLV (Gardner)	RD-114	CCC	Woolly	Gibbon	CCL-14.1	CCL-33	AMV	
S2Cl3	*100*	*57*	*100*	*48*	4.0	6.5	2.5	3.0	2.0	NT	*25*	*24*	5.0	2.5	1.4	NT
R-MuLV	*50*	*100*	*66*	*25*	NT	1.1	4.1	NT	NT	NT	NT	*11*	1.5	2.0	NT	NT
Ki-MuLV	*97*	*58*	*100*	*58*	4.0	6.0	0.2	NT	0.6	NT	*22*	*26*	6.0	1.8	1.6	1.2
CCL-38	7.0	NT	NT	NT	*70*	*100*	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
V-NRK	8.0	NT	6.0	NT	*100*	NT	NT	NT	NT	NT	NT	8.0	NT	NT	NT	NT
FeLV (Rickard)	2.6	NT	7.5	NT	2.8	3.0	*100*	*69*	1.2	5.0	NT	3.0	1.7	8.0	5.0	NT
FeLV (Gardner)	NT	NT	NT	NT	1.7	NT	*85*	*100*	2.8	4.4	NT	NT	NT	NT	NT	NT
RD-114	0.6	NT	1.6	2.0	1.4	NT	100§	100§	*100*	*75*	1.7	1.9	1.0	1.4	0.3	0.7
Woolly	*15*	*16*	*22*	*16*	2.0	2.5	1.2	NT	1.2	NT	*100*	*46*	0.4	0.2	2.0	NT
Gibbon	*25*	*20*	*27*	*16*	NT	2.5	1.6	1.7	0.7	NT	*49*	*100*	1.8	3.0	5.0	NT

* About 2000 cpm (0.07 pmol of [^{3}H]dTMP incorporated into DNA) of each [^{3}H]DNA product was hybridized to the RNA solutions. The percent hybridization values have been normalized with respect to the final percent hybridization obtained with the homologous viral RNA (actual values ranged from 50 to 80%). The values shown represent average final saturating percentage values obtained after addition of increasing amounts of viral RNA (up to 20 μg) or cytoplasmic RNA (up to 2 mg) extracted from a virus-producing culture. *NT*, not tested. *Italicized values* show greater than 10% homology.

† RNA extracted from purified virus preparations or from cells producing virus. Both sources of RNA yielded similar final saturating hybridization values.

‡ [^{3}H]DNA product prepared from the viruses. From some viruses (RD-114, R-FeLV, and Ki-MuLV) the DNA product was also prepared with isolated viral 60–70S RNA as a template for purified AMV RNA-dependent DNA polymerase (18); the results obtained were the same by both methods.

§ These values do not reflect an actual homology between RD-114 and the Rickard and Gardner–Arnstein strains of FeLV, but rather the presence of small levels of an endogenous feline virus homologous to RD-114 in the cat cell lines producing the two strains of FeLV (see Fig. 2 and *Discussion*).

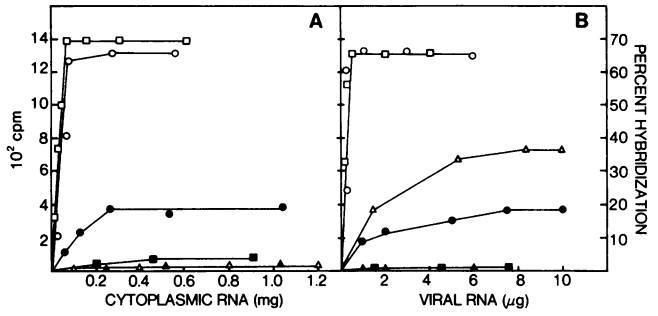

FIG. 1. Hybridization of murine [^{3}H]DNA products to RNA extracted from various type-C viruses. (*A*) The [^{3}H]DNA product (2000 cpm) was prepared from Ki-MuLV. Cytoplasmic RNA extracted from: (□) transformed NRK cells producing Ki-SV (Ki-MuLV); (○) S2Cl3 producer cells; (●) gibbon type-C virus growing in human SV clone 80 cells; (■) CCL-38, a rat-carcinoma cell line producing high titers of rat type-C virus; (▲) CCL-33, a pig-kidney cell line producing type-C virus; (△) SV clone 80 cells, SV40-transformed, apparently virus-free human cells. (*B*) The [^{3}H]DNA product (2000 cpm) was prepared from the S2Cl3 virus. Viral RNA extracted from: (□) Ki-SV (Ki-MuLV) virus; (○) S2Cl3; (△) R-MuLV; (●) woolly monkey type-C virus; (▲) avian myeloblastosis virus; (■) RD-114 virus. The radioactivity shown represents [^{3}H]DNA product that is resistant to digestion by nuclease S_1. Background levels [1–5% of the input radioactivity (19)] have been subtracted.

hamster, pig, primate, and avian type-C viruses were hybridized to either viral RNA or to cytoplasmic RNA extracted from cultures producing these viruses.

Within the feline type-C virus group, only RNA extracted from RD-114 or CCC virus hybridized significantly to the [^{3}H]DNA product prepared from RD-114 virus. RNA extracted from various murine, rat, primate, hamster, and pig type-C viruses, and from Mason–Pfizer monkey virus (20) hybridized to less than 2% of the RD-114 [^{3}H]DNA product. This DNA product is, therefore, specific only for endogenous feline type-C viral information. The Rickard and Gardner–Arnstein strains of feline leukemia virus, which are highly related to one another (69–85%), show no significant homology to either RD-114 or CCC virus or to the other type-C viruses, except for a small extent of homology (about 8%) with Ki-MuLV and with pig type-C viral RNA.

In the murine virus group, we examined S2Cl3, R-MuLV, Ki-MuLV, and AT-124 for homology. AT-124 was obtained from a human rhabdomyosarcoma cell (RD) that had been serially transplanted in immunosuppressed NIH Swiss mice (21). This virus possesses a group-specific antigen and an RNA-dependent DNA polymerase that are immunologically related to murine type-C viruses, and may thus be, in part or entirely, a type-C virus of NIH Swiss mice. The degree of homology between the viruses is shown in Table 2. S2Cl3 and Ki-MuLV cannot be distinguished by hybridization, and each of these probes shows a 50–60% homology to R-MuLV and AT-124. There is good agreement in the reciprocal hybridization experiments. The murine virus probes exhibit a low degree of homology (<6%) to cat, rat, hamster, pig, and avian type-C viral RNA, and to the RNA from the Mason–Pfizer virus. The two rat viruses examined (V-NRK and CCL-38) are highly homologous (70% related by hybridization) and show only a small degree of homology (<10%) to either murine or primate viruses.

A surprising degree of homology is apparent between all the murine type-C viruses and either of the primate type-C viruses (also see Fig. 1). The nucleic-acid probes of the two primate viruses are about 50% homologous and exhibit little nucleic-acid homology (<5%) to cat, rat, hamster, pig, and avian type-C viral RNA. However, they show a considerably greater extent of homology with each of the four mouse viruses; saturating values range from 15% with woolly monkey virus probe and endogenous BALB/c virus (S2Cl3) to 27% with the gibbon virus DNA probe and the RNA extracted from the Kirsten strain of mouse leukemia virus.

Table 3 shows the results of an experiment in which saturating concentrations of RNA extracted from cultures producing each of the primate viruses are hybridized simultaneously to S2Cl3 [^{3}H]DNA product. The same final percent hybridization value is obtained (19%) as when either viral RNA alone is added. There are, thus, closely related nucleic-acid sequences in the woolly and gibbon type-C viruses that are homologous to those of an endogenous mouse virus.

Detection of Two Cat Type-C Viruses in One Cell Line. The various feline leukemia virus (FeLV) isolates possess a common group-specific antigen (22, 23) and a common RNA-dependent DNA polymerase antigen (24) that distinguish this group of viruses from other mammalian type-C viruses. RD-114 has immunologic and host-range properties that are quite different from previously isolated feline type-C viruses (16, 25). A type-C virus can be induced from virus-free cell clones of the CCC continuous line of cat cells which has antigenic properties and host-range properties that are indistinguishable from those of RD-114 (25–27).

To examine the nucleic-acid homology between RD-114, CCC, and the two strains of FeLV, we prepared [^{3}H]DNA products from R-FeLV and from RD-114. The products were annealed to viral RNA extracted from these two viruses and from CCC. (Fig. 2). 0.25 μg of either RD-114 or CCC viral RNA saturates 55% of the [^{3}H]DNA product prepared from RD-114 virus. 0.25 μg of viral RNA extracted from R-FeLV hybridizes to 5% of the RD-114 [^{3}H]DNA product; however, a final level of 55% hybridization is achieved after addition of 16 μg of R-FeLV RNA. The most likely explanation for these

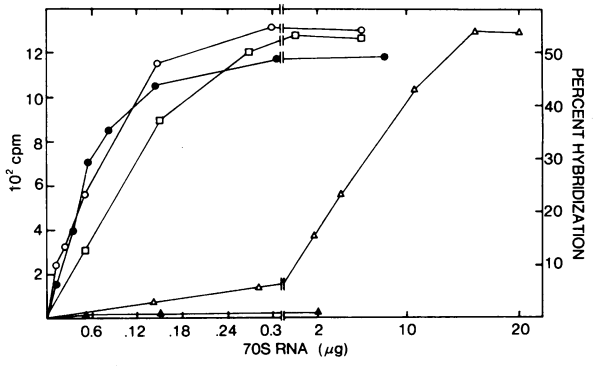

FIG. 2. Hybridization of feline type-C viral [^{3}H]DNA products to RNA extracted from RD-114, R-FeLV, and CCC viruses. 2400 cpm of [^{3}H]DNA product prepared from either RD-114 or R-FeLV was annealed to the viral RNAs as follows: (○) RD-114 [^{3}H]DNA and RD-114 RNA; (△) RD-114 [^{3}H]DNA and "R-FeLV" RNA; (□) RD-114 [^{3}H]DNA and CCC RNA; (●) R-FeLV [^{3}H]DNA and R-FeLV RNA; (▲) R-FeLV [^{3}H]DNA and RD-114 RNA.

data is that there are two viruses present in the cat cell line producing R-FeLV: the Rickard strain of feline leukemia virus and an endogenous cat virus that is related or homologous to RD-114. The [^{3}H]DNA product prepared from this mixture of viruses (the "R-FeLV" probe) hybridizes readily to RNA extracted from R-FeLV, but does not anneal to RD-114 viral RNA. Presumably only 2% of this DNA product would be RD or CCC specific, assuming both viral RNAs are copied symmetrically into DNA products in the endogenous RNA-dependent DNA polymerase reaction.

Table 4 lists further evidence for the presence of more than one type-C virus in a cell line. A [^{3}H]DNA product prepared from woolly type-C virus grown in human cells (A204) contains essentially no detectable information (1.0–1.5%) that is homologous to rat viral RNA, whereas 12–13% of a [^{3}H]-DNA product prepared from woolly monkey type-C virus grown in NRK cells contains information that is homologous to either CCL-38 or V-NRK RNA. Cytoplasmic RNA extracted from a rat cell line that is not producing virus (NRK clone 2) does not contain information that is homologous to either of these woolly monkey type-C virus DNA products.

DISCUSSION

The endogenous type-C viruses of mouse, rat, pig, hamster, and cat cells that we have examined exhibit very little, if any, nucleic-acid homology. Since endogenous viruses are believed to be transmitted vertically from cell to daughter cell as a part of their genetic material (28), their nucleic acids have probably evolved with the genetic material of their host organism and do not appear to be highly conserved.

A feline endogenous virus (CCC) is unrelated to either the Rickard or the Gardner–Arnstein strains of FeLV. A murine endogenous virus (S2C13), on the other hand, is very much related ($\geq$48% homology) to all the other murine viruses tested and cannot be distinguished from Ki-MuLV under our hybridization conditions. Therefore, an endogenous virus of a species may or may not be related to other viral isolates known to infect the same species.

Although the different infectious type-C virus isolates from one species consist of closely related viruses, in general there appears to be only a small degree of homology between the infectious type-C viruses of different species. The one notable exception is the 11–28% nucleic-acid homology observed between the various primate and murine viruses. In fact, the degree of homology between Kirsten mouse leukemic virus and gibbon ape virus probes (26 and 27%) is as great as that observed (25%) between two type-C viruses presumed to be of murine origin, Rauscher MuLV and AT-124. This large extent of primate–murine virus homology would not have been expected on an evolutionary basis. The difference between the genetic information of two endogenous vertebrate type-C viruses might reflect the extent of phylogenetic diversity between the two species (29, 30). Horizontally transmitted viruses, on the other hand, might exhibit a homology that would depend on the length of time since the species was infected. In addition, the infectious viruses could also change rapidly as the result of genetic recombination with type-C genomes in cells they infect. Thus, the homology between murine and primate viruses may reflect a recent infection of mice with an infectious primate virus, the infection of primates with a murine virus, or perhaps the infection of both species with a common "ancestor virus."

Because of the close homology between the primate viruses, they are potential probes for human type-C viral expression. If human and ape type-C viruses are indeed related, and also involved in tumors of primates, then the detection of putative type-C viral information in human tumors that is partially homologous to Rauscher MuLV (31) could be explained on the basis of the fortuitous murine–primate type-C virus homology. It is also possible that an endogenous human type-C virus will bear as little relationship to the known primate viruses as the two groups of feline type-C viruses exhibit towards each other.

The infection of a cell with one type-C virus can result in virus preparations that contain a mixture of viruses. Studies with type-C viruses that are produced in a heterologous cell line must, therefore, be interpreted cautiously. This has been most clearly demonstrated in those cell lines where the infecting virus and the endogenous virus are not closely related. Thus, cat cells infected with either the Rickard or the Gardner–Arnstein strain of FeLV can also produce the endogenous (CCC/RD-114) feline virus.

In the experiments described in this paper, we conclude that when primate type-C virus is grown in rat cells, the endogenous type-C virus of rat origin may account for up to 20% of the virus that is produced. If a general property of an infectious virus is the activation of an endogenous virus, this might be a useful method to obtain viruses of species from which the endogenous virus is not readily inducible. Two type-C

TABLE 3. *Common sequences in two primate type-C viruses detected by a murine viral DNA product*

RNA added (μg)*	% Hybridization with [^{3}H]DNA product from S2C13†
S2C13 (300)	71
Woolly monkey (600)	19
Gibbon ape (400)	20
Woolly monkey + gibbon ape (together, 1000)	19

* Cytoplasmic RNA extracted from cultures producing these viruses. The concentrations of RNA shown are in excess of the amount needed to saturate the S2C13 probe (see Fig. 1*A*).

† See legend to Table 2. The values shown represent actual hybridization values to the [^{3}H]DNA S2C13 product. Background hybridization values averaged about 50 cpm (2.5% of input radioactivity) and have been subtracted.

TABLE 4. *Detection of two distinct type-C viruses in rat NRK cells infected with woolly monkey type-C virus*

Percent hybridization*

[^{3}H]DNA \ RNA	V-NRK	CCL-38	NRK clone 2	Woolly/ A204	Woolly/ K-NRK
Woolly/ NRK	13	12	1.5	54	68
Woolly/ A204	1.0	1.5	1.0	70	65

* See legend to Table 2. The values shown represent final percent hybridization obtained after increasing concentrations of RNA were annealed to the [^{3}H]DNA products.

viruses, RD-114 and AT-124, have been isolated from the same human rhabdomyosarcoma cells after heterotransplantation into immunologically compromised animals. These viruses possess very few nucleic-acid sequences in common (Table 2). Although both viruses are grown in the same human cell line, they share very little cellular or putative endogenous human type-C information in common.

New virus isolates cannot be classified as belonging to a given species when there is extensive cross-species relatedness. Because of the high degree of nucleic-acid homology between the murine and primate viruses, the species of origin of AT-124 cannot be readily assigned. Thus, AT-124 viral RNA hybridizes to both murine and primate viral nucleic acids, although more extensively to the murine type-C viruses. AT-124, although most probably a murine virus, could still be a primate virus or a recombinant virus.

The RNA·DNA hybridization studies described here provide a basis for the classification of unknown viral isolates. A serious limitation to the hybridization studies described here involves the nonsymmetrical transcription of the viral RNA—the majority of the DNA product may represent only a small fraction (10%) of the viral genome (32). However, most of the type-C isolates of the various species exhibit a nucleic-acid diversity that parallels their evolutionary divergence. Several recent studies with cat cellular DNA have revealed a homology with RD-114; these studies led to the suggestion that RD-114 was, in fact, derived from cat cells (33–35). Based on the hybridization studies described here, RD-114 can be readily classified as an endogenous feline virus since it is highly homologous (70–90%) to the infectious type-C viruses that can be induced from cat cell clones in culture (25–27).

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1. Todaro, G. J. & Huebner, R. J. (1972) *Proc. Nat. Acad. Sci. USA* **69,** 1009–1015.
2. Todaro, G. J. (1973) *Biohazards in Virus and Cancer Research,* eds. Hellman, A., Oxman, M. & Pollock, R. (Cold Spring Harbor Press, New York), pp. 114–130.
3. Aaronson, S. A., Hartley, J. W. & Todaro, G. J. (1969) *Proc. Nat. Acad. Sci. USA* **64,** 87–94.
4. Vogt, P. K. & Friis, R. R. (1971) *Virology* **43,** 223–234.
5. Lowy, D. R., Rowe, W. P., Teich, N. & Hartley, J. W. (1971) *Science* **174,** 155–156.
6. Aaronson, S. A., Todaro, G. J. & Scolnick, E. M. (1971) *Science* **174,** 157–159.
7. Scolnick, E. M., Parks, W. P. & Livingston, D. M. (1972) *J. Immunol.* **109,** 570–577.
8. Oroszlan, S., White, M., Gilden, R. V. & Charman, H. A. (1972) *Virology* **50,** 294–297.
9. Parks, W. P., Scolnick, E. M., Ross, J., Todaro, G. J. & Aaronson, S. A. (1972) *J. Virol.* **9,** 110–115.
10. Scolnick, E. M., Parks, W. P., Todaro, G. J. & Aaronson, S. A. (1972) *Nature* **235,** 35–40.
11. Haapala, D. K. & Fischinger, P. J. (1973) *Science* **180,** 972–974.
12. Todaro, G. J. (1972) *Nature* **240,** 157–160.
13. Duc-Nguyen, H., Rosenblum, E. N., & Zeigel, R. F. (1966) *J. Bacteriol.* **92,** 1133–1140.
14. Aaronson, S. A. & Weaver, C. A. (1971) *J. Gen Virol.* **13,** 245–252.
15. Todaro, G. J., Wolman, S. R. & Green, H. (1963) *J. Cell. Physiol.* **62,** 257–265.
16. McAllister, R. M., Nicholson, M., Gardner, M. B., Rongey, R. W., Rasheed, S., Sarma, P. S., Huebner, R. J., Hatanaka, M., Oroszlan, S., Gilden, R. V., Kabigting, A. & Vernon, L. (1972) *Nature* **235,** 3–6.
17. Ross, J., Tronick, S. & Scolnick, E. M. (1972) *Virology* **49,** 230–235.
18. Benveniste, R. E., Todaro, G. J., Scolnick, E. M. & Parks, W. P. (1973) *J. Virol.* **12,** 711–720.
19. Benveniste, R. E. & Scolnick, E. M. (1973) *Virology* **51,** 370–382.
20. Chopra, H. C. & Mason, M. M. (1970) *Cancer Res.* **30,** 2081–2086.
21. Todaro, G. J., Arnstein, P., Parks, W. P., Lennette, E. H. & Huebner, R. J. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 859–862.
22. Hardy, W. D., Jr., Geering, G., Old, L. J., DeHarven, E., Brodey, R. S. & McDonough, S. (1969) *Science* **166,** 1019–1021.
23. Oroszlan, S., Huebner, R. J. & Gilden, R. V. (1971) *Proc. Nat. Acad. Sci. USA* **68,** 901–904.
24. Scolnick, E. M., Parks, W. P. & Todaro, G. J. (1972) *Science* **177,** 1119–1121.
25. Livingston, D. M. & Todaro, G. J. (1973) *Virology* **53,** 142–151.
26. Fischinger, P. J., Peebles, P. T., Nomura, S. & Haapala, D. K. (1973) *J. Virol.* **11,** 978–985.
27. Sarma, P. S., Tseng, J., Lee, Y. K. & Gilden, R. V. (1973) *Nature New Biol.* **244,** 56–59.
28. Huebner, R. J. & Todaro, G. J. (1969) *Proc. Nat. Acad. Sci. USA* **64,** 1087–1094.
29. Martin, M. & Hoyer, B. H. (1966) *Biochemistry* **5,** 2706–2713.
30. Kohne, D. E. (1970) *Quart. Rev. Biophys.* **33,** 327–375.
31. Hehlmann, R., Kufe, D. & Spiegelman, S. (1972) *Proc. Nat. Acad. Sci. USA* **69,** 435–439.
32. Garapin, A. C., Varmus, H. E., Faras, A. J., Levinson, W. E. & Bishop, J. M. (1973) *Virology* **52,** 264–274.
33. Baluda, M. A. & Roy-Burman, P. (1973) *Nature New Biol.* **244,** 59–62.
34. Neiman, P. E. (1973) *Nature New Biol.* **244,** 62–64.
35. Ruprecht, R. M., Goodman, N. C. & Spiegelman, S. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 1437–1441.
36. Armstrong, J. A., Porterfield, J. S. & deMadrid, A. T. (1971) *J. Gen Virol.* **10,** 195–197.