Some Biological Properties of Two New Avian Sarcoma Viruses

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The new avian retroviruses UR1 and UR2 were isolated from spontaneous tumors of chickens by cocultivation of tumor material with susceptible chicken embryo fibroblasts. In vitro, UR1 induced formation of small foci of round and fusiform cells. On the other hand, cells infected by UR2 assumed an extremely elongated morphology. In vivo, both viruses induced fibrosarcomas and myxosarcomas with short latencies. Infectivity assays with and without mitomycin C showed that both viruses were defective for replication, but transformed nonproducing cell clones were obtained only with UR1. UR1-infected transformed nonproducing clones did not release particles detectable by reverse transcriptase assays, and fusion of transformed nonproducing cells with quail cells chronically infected with Rous sarcoma virus (a Bryan strain) failed to rescue infectious virus. This suggested that UR1 does not code for functional envelope glycoproteins. In this regard, UR1 appeared to be similar to Fujinami, PRCII, and Y73 viruses. The helper viruses of partially purified stocks of UR1 and UR2 appeared to belong to subgroup A, but these helper viruses were distinguishable from each other, as shown by host range experiments and neutralization tests. Hybridization studies with DNA complementary to the *src* gene of Rous sarcoma virus and RNAs extracted from both UR1 and UR2 showed no homology between the genomes of the new isolates and the transforming gene of Rous sarcoma virus.

Avian sarcoma viruses produce solid tumors in animals and cause transformation of susceptible cells in vitro. For several laboratory strains of Rous sarcoma virus (RSV) (16) and for Bratislava B77 sarcoma virus, an independent virus isolate from Czechoslovakia (24), neoplastic transformation has been shown to be related to the presence of the viral gene *src*, which is located at the 3' end of the virus RNA genome (26, 27) and codes for a 60,000-dalton protein, pp60 (3). Recent investigations of Fujinami sarcoma virus (5) and sarcoma virus PRCII (4) showed that these viruses do not contain sequences related to *src* but do contain a new transforming insertion designated *fps*, which is located at the middle of the genome and is fused to the gene which codes for viral structural proteins, *gag* (2, 8, 13, 17). Moreover, Kawai et al. found that another newly isolated sarcoma virus, Y73 (10), contains transforming information (*yas*) which is not related to either *src* or *fps* (12). Sequences related to these viral transforming genes have been identified in cells of a num-

ber of vertebrate species (19, 20, 29; Shibuya, unpublished data). It has been suggested that these transforming viruses might have arisen when an avian retrovirus (avian lymphoid leukemia virus) acquired cellular genes through recombination (7, 19). Therefore, it appears that the study of additional independent virus isolates might lead to the identification of other cellular genes which are potentially oncogenic for vertebrate cells.

This report describes some of the biological properties of two previously undescribed viruses, which were isolated from tumor materials originally obtained as spontaneous tumors in chickens in the United States in 1963 and 1969. These viruses, designated UR1 and UR2, are defective in replication and appear to contain transforming insertions unrelated to *src*.

MATERIALS AND METHODS

Viruses. The origin and preparation of UR1 and UR2 are described below. Other viruses used in this investigation were Rous-associated virus-1 (RAV-1) and RAV-3, two subgroup A leukemia viruses (originally from P. K. Vogt), RAV-6 (subgroup B; kindly provided by R. Dougherty), RAV-7 (subgroup C; a gift

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from R. Weiss), and RAV-50 (subgroup D; received from H. Hanafusa). RAV-1 was used to rescue transforming virus from nonproducing cell clones infected with UR1. The other viruses mentioned above were used in interference experiments. RSV Prague strain subgroups A and C (originally from J. Wyke), Bryan strain RSV [BH-RSH(RAV-6)] subgroup B, and Schmidt-Ruppin RSV subgroup D were used as controls in host range experiments.

Cells and media. Chicken embryo cells of phenotype C/E were derived from leukosis-free embryos obtained from Spafas, Norwich, Conn. Eggs of genotype C/C from line 15 and eggs of genotypes C/A and C/ABE from the F1 progeny of a cross between line 100B and line 7₂ were obtained from the Regional Poultry Laboratory, U.S. Department of Agriculture, East Lansing, Mich. Cells from each embryo were tested for susceptibility to subgroup A through D avian sarcoma viruses and for absence of chicken helper factor. Cell cultures were maintained in Eagle minimal essential medium containing 10% tryptose phosphate broth, 4% calf serum, penicillin (100 IU/ml), and streptomycin (100 µg/ml). Transformed chicken cells (both producers of infectious virus and nonproducers) were maintained in the same medium supplemented with 0.5% dimethyl sulfoxide. Turkey cells from embryonated eggs were obtained from Timmerman Farms, Inc., La Fargeville, N.Y., and were maintained in the same medium supplemented with 1% turkey serum. Japanese quail cells (a gift from C. Moscovici) were maintained in Ham F10 medium containing 10% tryptose phosphate broth and 4% calf serum. Line 16Q, a line of quail cells chronically infected with BH-RSV(-), was obtained from H. M. Murphy (14) and was grown in Dulbecco modified minimal essential medium containing 10% tryptose phosphate broth, 4% fetal calf serum, and 1% dimethyl sulfoxide.

Infectivity assays were performed by standard procedures (25). Infectious center assays with mitomycin C were performed by the method of Weiss et al. (28). Briefly, infected cells on 60-mm plates were treated with mitomycin C (2 µg/ml) 24 h postinfection. After overnight exposure, the cultures were washed twice, and fresh medium was added for 2 h. After this medium was removed, 8×10^5 C/E indicator cells were added, and the plates were overlaid with agar medium 4 to 5 h later.

Interference tests were performed by previously described methods (18). Cloning of transformed nonproducing (TNP) cells was carried out by picking foci of transformed cells through agar and growing these foci in plates (Costar) with 16-mm wells containing 1 ml of an enriched medium consisting of 86 ml of Ham F10 medium, 12 ml of calf serum, 14 ml of tryptose phosphate broth, 4.5 ml of chicken serum, 1.2 ml of a minimal essential medium-100× vitamin mixture, 1.2 ml of folic acid (0.8 mg/ml), and 1 ml of dimethyl sulfoxide. When the cell growth became confluent, the cultures were transferred successively to 35-mm plates, 60-mm plates and, when required, 100-mm plates.

Reverse transcriptase assay. Reverse transcriptase activity was measured directly from unconcentrated tissue culture fluids. Portions of infected cell

media (or uninfected cell media as controls) were clarified with a Brinkman microcentrifuge, and 50-µl samples were incubated for 2.5 h at 40°C with 50 µl of the standard reaction mixture. This mixture contained 50 mM Tris-hydrochloride (pH 8.0), 50 mM KCl, 10 mM dithiothreitol, 10 mM MgCl₂, 0.5% Triton X-100, 5 µg of polyadenylic acid-oligothymidylic acid₁₂₋₁₈, and 20 µM [*methyl*-³H]TTP (5 µCi of 30 Ci/mmol; adjusted with unlabeled TTP; Amersham Corp., Arlington Heights, Ill.).

The reactions were terminated by rapid cooling on ice. Each reaction mixture was transferred to Whatman no. 3 filter paper. Filters were batch washed through a series of 5% trichloroacetic acid baths (15), dried, and counted with a liquid scintillation spectrometer.

Rescue of infectious virus with RAV-1. Cells from TNP clones were cocultured with C/E cells previously infected with RAV-1. These cultures were propagated for one or two passages before the supernatant fluids were tested for infectious transforming virus after two cycles of freezing and thawing. Occasionally, TNP cells were fused with RAV-1-infected cells by using polyethylene glycol; for this we used the procedure described by Steimer and Boettiger (21). This procedure was also used in an attempt to complement the *env* defect of BH-RSV(-) by fusing 16Q cells with TNP cells infected with UR1 virus.

Preparation of DNA complementary to viral RNAs. [³H]DNA complementary to the *src* gene of RSV (cDNA_{src}) was prepared as single-stranded DNA synthesized by using avian myeloblastosis virus reverse transcriptase in a reaction containing 39S RNA from Schmidt-Ruppin RSV (subgroup B) and calf thymus DNA primers (22). The procedures used for the synthesis of cDNA and for the selection of cDNA_{src} with RAV-2 RNA were essentially as described previously (17). The preparation of [³H]DNA complementary to Fujinami-associated virus (cDNA_{FAV}) has been described previously (17).

RESULTS

Primary source of the new isolates and isolation procedure. The transforming viruses described here were isolated from tumor material provided by B. W. Calnek (Cornell University, Ithaca, N.Y.) and R. E. Luginbuhl (University of Connecticut, Storrs) and were designated UR1 and UR2, respectively, to indicate that they were isolated at the University of Rochester.

The primary tumor submitted by Calnek appeared in a strain S chicken of unknown age derived from a Poultry Department flock at Cornell University in 1969. The tumor observed by Luginbuhl was originally found in a 36-week-old White Rock chicken submitted to the University of Connecticut Poultry Diagnostic Laboratory in 1963. A postmortem examination revealed adenomas throughout the entire abdominal cavity. Histological examinations of the kidney, pancreas, and intestine revealed encapsulated myxofibromas. The pancreas of the bird

was triturated with TC medium 199, and the resulting preparation was inoculated into 8-day-old Spafas chickens via intraperitoneal, intramuscular, and wing web routes. Tumors appeared in some of the birds in each group.

In 1970, tumor suspensions made from frozen materials provided by Calnek and Luginbuhl were injected by one of us (H.R.M.) into the wing webs of a number of chickens; some of these chickens developed tumors, whereas several attempts to isolate the viruses directly from the original material by inoculation into cultures of C/E cells failed. When cell suspensions obtained by trypsinization from some of the secondary tumors from the two origins were passed in vitro with C/E chicken embryo fibroblasts, many cells became morphologically transformed. The transforming ability of the cell culture fluids could be transferred serially in C/E chicken embryo fibroblasts.

Virus purification. Supernatant fluids from cultures infected with UR1 and UR2 were inoculated at several dilutions onto C/E cells which were then overlaid with agar medium. After 7 to 8 days foci of cells with altered morphology were observed.

The foci of transformed cells in the plates infected with UR1 were rather uniform in size and ranged from 1 to 2 mm in diameter. The cells in these foci were very refractile, enlarged, and globular in shape. In some foci, unusually large cells were observed. Many of these cells contained vacuoles. Intermixed with the round cells or, more often, at the periphery of foci, refractile spindle-shaped cells were found. These cells did not contain vacuoles (Fig. 1A).

The foci in the plates infected with UR2 ranged in size from 2 to 4 mm and were quite different from the foci caused by UR1 (Fig. 1B). These foci were made up of very tight bundles of cells having an accentuated fibroblastic morphology and often arranged to give a triangular appearance to the foci. Overall, these foci resembled the foci found with the morph^f variants of BH-RSV (23). However, they were less distinct and hard to see with the naked eye. In order to count these foci, we found it convenient to remove the agar from the plates, rinse the monolayers quickly with Tris buffer, stain the cultures for 30 s to 1 min with 0.5% methylene blue in water, and destain them briefly with 50% methanol, according to the procedure by Barlati et al. (1). Foci of transformed cells acquired a blue color, which stood clearly against the unstained background of normal cells (Fig. 1C).

The supernatant fluids assayed in these early experiments had titers of 3.8×10^4 and 2.9×10^4 focus-forming units per ml for UR1 and UR2,

FIG. 1. (A) Morphology of cells transformed by UR1. Bar = 100 μ m. (B) Morphology of cells transformed by UR2. Bar = 100 μ m. (C) Focus of turkey cells transformed by UR2 and stained with 0.5% methylene blue. Bar = 250 μ m.

respectively. In later passages higher titers were obtained. When these materials were inoculated into plates of C/E, C/ABE, C/C, and turkey cells, foci of transformed cells with their typical morphology developed in approximately the same number in all cell types, with the exception of the C/ABE plates, which had considerably fewer foci. These results suggested that the virus

stocks were not pure and presumably contained different pseudotypes of the same transforming viruses. To purify the transforming agents and to obtain virus of a single envelope type for further investigations, several dilutions of each virus stock were inoculated into 60-mm plates of C/E cells and overlaid with agar medium. Transformed cells in single foci were isolated from plates with less than 10 foci.

A few virus stocks derived from single foci were tested for host range in C/E, C/A, C/ABE, C/C, Japanese quail, and turkey cells (Table 1). C/C cells appeared to be the most susceptible cells to both UR1 and UR2 purified stocks. C/A and C/ABE cells were completely resistant. Quail cells were susceptible to the purified stocks of UR1, which formed well-defined foci, but they were rather resistant to the UR2 stocks, which formed a few minute indistinct foci of altered cells. On the other hand, turkey cells were very susceptible to the UR2 stocks, which formed small but well-defined foci. The foci of UR1 in turkey cells were as numerous as those in quail cells but were very indistinct.

The results of these experiments suggested that the purified stocks of UR1 and UR2 tested belonged to subgroup A, but their relative susceptibilities in quail and turkey cells made them atypical. Interference experiments with avian leukosis viruses belonging to subgroups A through D showed that only cells preinfected with RAV-3, a subgroup A leukosis virus, were resistant to infection with UR1 and UR2 purified stocks (data not shown). Also, studies with immune sera prepared with viruses belonging to subgroups A through D and with purified stocks of UR1 and UR2 confirmed that the latter viruses belonged to subgroup A, but revealed antigenic differences between UR1 and UR2 (data not shown). Overall, the data from these studies showed that the viruses in these stocks were subgroup A viruses. We made no further at-

tempts to identify additional pseudotypes of the transforming agents, and the following studies were performed with cloned subgroup A viruses.

Defectiveness of UR1. To determine whether UR1 was defective for replication, a dilution experiment with and without mitomycin was performed. Briefly, a virus stock from a purified clone was diluted in 0.5-log increments and inoculated into two sets of plates. One of these sets was overlaid with agar medium after 45 min of adsorption for a focus-forming assay. The other set was exposed for 18 h to 2 μ g of mitomycin C per ml beginning 24 h after infection and was subsequently treated as described above for an infectious center assay. Figure 2A shows the estimated titer relative to the titer calculated for the lowest dilution for both the focus-forming assay and the infectious center assay. This figure shows that the estimated titer for the focus-forming assay was rather constant over a range of dilutions between 10^{-2} and $10^{-3.5}$. On the other hand, the titer for the infectious center assay decreased considerably over the same dilution range. This indicated that the transforming virus was replication defective. On the basis of this information, we attempted to isolate nonproducing clones of transformed cells. A few purified clones were inoculated at different dilutions. After 6 h the cultures were overlaid with agar medium containing antiserum made against uncloned UR1. A total of 30 foci were picked from plates containing 10 foci or less, and these foci were transferred to Costar plates containing 1 ml of enriched medium per well. When the cells reached confluency, they were transferred to 35-mm plates. Morphologically, the cells derived from individual clones were either clearly transformed (18 of 30) or were similar to normal fibroblasts. Supernatant fluids from confluent 35-mm plates were tested for infectivity and reverse transcriptase activity. Of the obviously transformed cultures, nine were negative

TABLE 1. *Host ranges of original stocks and purified stocks of UR1 and UR2 and other viruses*

Virus	Titer on: ^a					
	C/E cells	C/A cells	C/ABE cells	C/C cells	Quail cells	Turkey cells
UR1 original stock	27	28	1	100	16	12
UR1 stock 7	58	0	25	100	75	77
UR2 original stock	45	ND ^b	7	100	5	95
UR2 stock 5	43	0	0	100	2	85
UR2 stock 12	37	0	0	100	10	90
Prague RSV (subgroup A)	27	0	0	74	47	100
BH-RSV(RAV-6) (subgroup B)	71	71	0	100	0	0
Prague RSV (subgroup C)	71	28	38	0	8	100
Schmidt-Ruppin RSV (subgroup D)	49	33	0	100	0	0

^a Titers are expressed as percentages of focus-forming units present in the most sensitive cell type.

^b ND, Not done.

for infectivity and also for reverse transcriptase activity. It was interesting that the transformed producing cultures of clonal origin could be maintained in good condition for five to six successive transfers, whereas TNP cultures deteriorated quite rapidly. The reasons for this behavior are not known. From seven of nine such TNP clones, transforming subgroup A virus could be rescued after coculture with RAV-1-infected cells. To determine whether, in addition to the defect in the polymerase gene, UR1 also had a defect in the *env* gene (which codes for envelope glycoproteins gp85 and gp37), we attempted to fuse TNP cells with 16Q cells which were chronically infected with BH-RSV(-). Since BH-RSV(-) possesses a functional polymerase, codes for virion structural proteins, and is missing most or all of the *env* gene (11), we expected that infectious transforming virus would be produced if UR1 were able to code for a functional *env* product. The fusion was performed with polyethylene glycol by the method of Steimer and Boettiger (21). Rescue of BH-RSV(-) was not observed in any of these experiments, whereas fusion of 16Q cells with a producer clone of UR1-transformed cells complemented BH-RSV(-), as shown by the presence of virus able to cause the typical BH-RSV transformation in the supernatant of fused cultures. These results suggested that UR1 is defective in both *env* and *pol* genes.

Defectiveness of UR2. Dilution experiments to investigate the possible defectiveness of UR2 were performed as described above. Of the two purified stocks tested, (stocks 5 and 12), only one (stock 5) gave an estimated titer in the infectious center assay which was compatible with a replication defect of this virus (Fig. 2B). The other stock gave estimated titers for both infectious center and focus-forming assays which were indistinguishable (data not shown). These results suggested that either the transforming UR2 virus is indeed defective and that in some clones a large excess of helper virus is present or that UR2 is a mixture of nondefective and defective viruses. When stock 12 virus was tested at limiting dilutions for transformation and reverse transcriptase activity, we found that reverse transcriptase-positive helper virus was present in at least 100-fold excess (data not shown). Therefore, these data confirmed the first assumption. However, a search for TNP clones performed on more than 85 foci picked through agar has been unsuccessful so far.

Studies in vivo. Undiluted supernatant fluids from cell cultures infected with purified virus stocks containing both UR1 and UR2 and their original subgroup A helper viruses (ap-

FIG. 2. Focus-forming assays (FFA) and infectious center assays (ICA) of UR1 (A) and UR2 (B). Dilutions of UR1 and UR2 were tested in two sets of plates. One set was overlaid with agar medium 45 min after infection for conventional focus-forming assays. The other set was treated with mitomycin C (2 μ g/ml) at 24 h postinfection. After 18 h the cultures were washed thoroughly, and 8×10^5 indicator cells were added to the plates, which were overlaid 5 h later for infectious center assays.

proximately 10^5 focus-forming units per ml) were injected into the wing webs of several 4-week-old White Leghorn chickens from the Spafas flock. When 0.2- to 0.4-ml volumes were injected, palpable tumors could be detected as early as 8 days after inoculation with UR1. Birds sacrificed 18 days after UR1 inoculation had tumors 1 to 2 cm in diameter. These tumors were of two types; they were either firm nodular tumors about 1 cm in diameter or soft tumors about 2 cm diameter. Sometimes the two components (soft and firm) were present in the same tumor. Microscopically, the soft and hard tumors were rather similar, the most significant difference between the two being the relative predominance of a mucoid edematous component in the soft tumors and the presence of prominent lymphoid nodular components within the hard tumors (Fig. 3A through C). The tumors themselves were composed of areas of polygonal cells with large clear nuclei and one or two nucleoli; we found one to three mitotic figures per high-power field ($\times 400$) and areas showing a spindle cell type of tumor, sometimes infiltrating the periphery of skeletal muscle. The spindle type of tumor was either very compact with several mitotic figures or loose with cells rather dispersed in a mucoid matrix.

In birds infected with UR2, tumors appeared somewhat late. The earliest tumor did not appear until 2 weeks after inoculation. Microscopic examinations of tumors in birds sacrificed 1 week after tumor appearance showed both compact and edematous spindle cell sarcomas but no masses of round or polygonal cells. Also, within the tumors there was a prominent lymphocytic nodular component with obvious germinal centers in many of the nodular aggregates. There

FIG. 3. (A) Areas of different cell densities in a tumor caused by UR1. Low magnification. (B) Area of high cell density in a tumor caused by UR1. The cells are predominately round or polygonal. High magnification. (C) Round and polygonal cells interspersed in a mucoid matrix in a tumor caused by UR1. (D) Areas of high and low cell densities in a tumor caused by UR2. (E) Area of high density in a tumor caused by UR2. High magnification. Fusiform cells are arranged in bundles. (F) Lymphoid infiltrate in a tumor caused by UR2. High magnification.

were also many lymphocytes scattered throughout the tumors and clear invasions of tumor cells into adjacent skeletal muscles, with destruction of muscle fibers.

In summary, the tumors caused by UR1 and UR2 were not dissimilar from the myxosarcomas caused by other avian sarcoma viruses.

Search for *src*. Since many properties of

UR1 and UR2, including the ability to cause morphological conversion of cells in vitro and the ability to induce myxosarcomas in vivo, were similar to RSV properties, we investigated whether the transforming abilities of these agents were due to the presence in their genomes of sequences homologous to the *src* gene of RSV.

Viruses from cloned purified stocks which had

less than a fivefold excess of helper virus over transforming virus, as determined by transformation and reverse transcriptase assays, were sedimented by centrifugation at 19,000 rpm for 2 h after clarification of supernatant fluids. Viral RNA was extracted from each pellet with phenol-chloroform-isoamyl alcohol (1:1:0.01) and 1% sodium dodecyl sulfate. Hybridization experiments with viral RNA and a *src*-specific cDNA prepared with Schmidt-Ruppin RSV or Fujinami-associated virus-specific cDNA were performed by previously described methods (8). These experiments (Table 2) indicated that although extensive hybridization occurred between the RNA of UR1 or UR2 and cDNA_{FAV}, no significant hybridization took place with cDNA_{src}.

Thus, the genomes of UR1 and UR2 do not contain sequences homologous to the *src* gene of RSV.

DISCUSSION

Since the first isolation of an avian sarcoma virus by Rous in 1911 (16), reports of other isolates have been few and far between. In 1914 Fujinami and Inamoto reported the isolation of a second avian sarcoma virus with properties similar to those of RSV (5), but it was not until 1958 that another isolate (PRCII) was reported by Carr and Campbell (4). In 1963 Thurzo et al. isolated the B77 strain of avian sarcoma virus (24), and, more recently, Itohara et al. (10) reported the isolation of avian sarcoma virus Y73. Thus, the new viruses which we describe here represent a significant addition to the few known avian viruses that are able to cause solid tumors in vivo. These two agents appear to be interesting in many respects. Although the tumors produced by these viruses in chickens do not appear to be different from the fibrosarcomas and myxosarcomas induced by the previously reported agents, the types of morphological conversions which they produce in vitro are sufficiently different from those of other isolates to be considered unique. Foci of UR1 are characterized by their small size and by a considerable enlargement of the transformed cells. On the other hand, UR2 causes an accentuated elongation of cells reminiscent of the morph⁺ type of transformation caused by variants of BH-RSV and Fujinami virus. However, although the latter agents occasionally mutate to a morph⁺ type of transformation (23), with UR2 a change in the type of morphological conversion has not been observed so far. Thus, the transforming gene of this virus appears to be very stable.

A comparison of focus-forming assays and infectious center assays with mitomycin indicated

TABLE 2. Absence of the *src*-specific sequences in UR1 and UR2 RNAs^a

Viral RNA	Extent of hybridization (%) with:	
	cDNA _{src}	cDNA _{FAV}
Schmidt-Ruppin RSV (subgroup B)	93	56
UR1 + helper	1	69
UR2 + helper	1	74
RAV-2	1	60
Fujinami-associated virus	2	87

^a Varying amounts (0.1 to 5 μ g) of viral RNAs were mixed with [³H]cDNA_{src} (400 cpm) or [³H]cDNA_{FAV} (600 cpm) and hybridized under conditions of moderate stringency (50°C in 30% formamide–0.45 M NaCl–0.045 M sodium citrate [pH 7.4]–5 mM EDTA–0.4% sodium dodecyl sulfate). The extent of hybridization was measured by S1 nuclease digestion (9). The hybridization reaction was performed at a C_t value of 2 mol·s/liter, based on total viral RNA concentration. The C_t_{1/2} value for the cross-hybridization between UR1 plus helper virus or UR2 plus helper virus RNA and cDNA_{FAV} was about 0.02 mol·s/liter.

that both UR1 and UR2 are defective in replication.

Therefore, the subgroup A purified virus stocks, as determined by host range interference experiments and neutralization tests, must be the subgroup of the helper viruses which formed pseudotypes of UR1 and UR2. It is interesting that the relative efficiencies of focus formation by UR1 and UR2 in quail and turkey cells (both susceptible to subgroup A viruses) are different. This deserves further investigation.

Attempts to isolate TNP clones from UR2 have been unsuccessful. Most likely, a large excess of helper virus present in the stocks causes double infections of individual UR2 foci.

On the other hand, isolation of TNP clones has been achieved with UR1. Supernatant fluids from TNP clones do not contain reverse transcriptase activity, and experiments on the fusion of TNP cells with 16Q cells carrying the replication-defective RSV(–) suggest that UR1 also lacks a functional gene coding for envelope glycoproteins. From this point of view, UR1 is similar to Fujinami virus, PRCII, and Y73 and different from RSV(–) and NY8 (11). The latter two viruses are defective for replication but code for reverse transcriptase and presumably were derived from originally nondefective avian sarcoma virus (6).

Preliminary investigations of the transforming insertions of UR1 and UR2 were particularly interesting. Our data show that there is no homology between the cDNA specific to the *src* gene of RSV and the RNAs extracted from UR1

and UR2 viruses. Further studies will reveal whether the transforming insertions of these viruses are homologous to other known transforming genes or are new.

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ADDENDUM IN PROOF

UR2 transformed, nonproducing clones have been obtained and virus could be rescued with a number of Rous-associated viruses.

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