A Mutant of Rous Sarcoma Virus (Type O) Causing Fusiform Cell Transformation[1] (35039)

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(Introduced by R. W. Schlesinger)

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The interaction between Rous sarcoma virus (RSV) and cell is determined by viral phenotype and genotype. The best studied phenotypic properties of RSV are those belonging to the viral envelope. They affect viral host range, sensitivity to interference with avian leukosis viruses and interaction with neutralizing antibody. On the other hand, cellular transformation appears to be dependent on expression of the viral genome. In the course of transformation the virus influences cellular morphology as well as social behavior, and various genetic strains of RSV differ in their modification of host cell properties (1–4). The most conspicuous of these differences is that between RSV which causes the transformed cells to become spherical and RSV which bestows a fusiform shape on the cell.

The Bryan high-titer strain of RSV commonly occurs in the form of pseudotypes which are the result of excessive phenotypic mixing with avian leukosis viruses (5). From such pseudotype stocks an RSV has been derived which causes round cell transformation and no longer contains overt avian leukosis viruses (6, 7). This agent is known as RSV type O. An agent inducing exclusively fusiform cell transformation was also derived from the Bryan high-titer strain of RSV (1, 2). Available preparations of this fusiform RSV are likely to consist of pseudotype virus because they also contain high titers of nontransforming avian leukosis viruses. Therefore, it was suspected that the true nature of fusiform RSV had been obscured by phenotypic mixing with avian leukosis viruses. The present paper confirms this assumption and describes the isolation of an RSV which induces fusiform cell transformation and is free of overt avian leukosis viruses.

Materials and Methods. Virus. A stock of Bryan high-titer RSV containing Rous-associated virus type 3 [RSV (RAV-3)] was observed to produce a small fraction of foci with fusiform cell morphology. A purely fusiform line of RSV was derived from RSV(RAV-3) by several successive single-focus isolations. The fusiform stock was antigenically identical to RAV-3 and contained RAV-3 as an associated agent. From this stock a leukosis-free RSV was isolated as described below. It will be referred to as fRO, for fusiform RSV type O. The original type O RSV causes round cell transformation and will be abbreviated rRO. Neither fRO nor rRO belong to an established avian tumor virus subgroup (6, 7). Origin and production of the following viruses have been described previously: Rous-associated virus (RAV) types 1 and 5 (subgroup A), RAV-2 (subgroup B), RAV-7 (subgroup C), Carr–Zilber-associated virus (CZAV), and RAV-50 (subgroup D) (8, 9).

Cell cultures and virus assay. Source and susceptibility patterns of the avian cell types used have been described (9). The assay of RSV was carried out in the presence of 2 μg/ml polybrene (10).

Immunological techniques. Virus neutrali-

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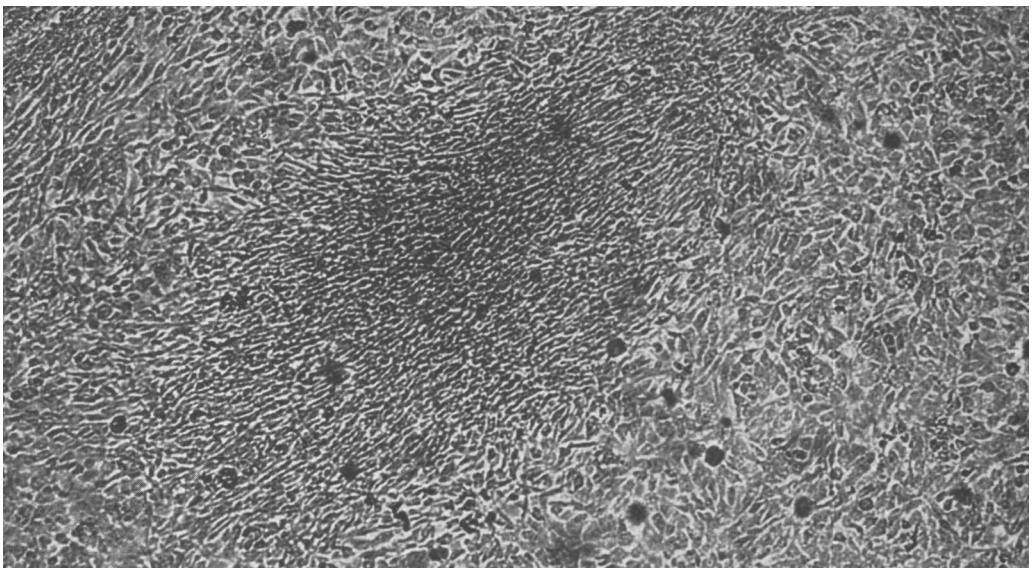

FIG. 1. Type C/A chick embryo fibroblast culture with a focus induced by fRO.

zation by antibody was determined by assaying for surviving focus formers after incubation at 37° for 40 min with appropriate serum dilutions. Fluorescent staining of infected cells was carried out without prior fixation. The details have been described (11, 12).

Results. Isolation of a leukosis-free Rous sarcoma virus causing fusiform cell transformation. Type C/B chick embryo fibroblasts were infected with a high dilution of fusiform RSV(RAV-3) yielding from 3–5 foci per 60-mm dish. The cultures were overlaid on the next day with nutrient agar containing a dilution of chicken serum sufficient to neutralize more than 99% of fusiform RSV(RAV-3). On day 7 foci were marked with a wax pencil, aspirated through the agar into the tip of a capillary pipette, and transferred onto normal C/B feeder cells. The plates containing isolated single foci were then transferred at 2- to 3-day intervals, and the growth medium was tested for the presence of free virus before each transfer. Of 110 foci tested 109 produced fusiform RSV of the parental serotype. One failed to release virus capable of transforming type C/O or C/B chick embryo fibroblasts but unlike the parental virus did produce foci on type C/A cells. This host range is characteristic of rRO, and the observations described in this paper indicate that the new viral isolate is a mutant of rRO causing fusiform cell transformation. It will be referred to as fRO. The foci induced by fRO are illustrated in Fig. 1.

A stock preparation of fRO was grown in type C/A chick embryo fibroblasts and was tested for the presence of an avian leukosis virus by searching for an agent capable of interfering with focus formation by fRO (13). Two such tests failed to detect avian leukosis virus beyond the endpoint of transformation by fRO. This indicated that preparations of fRO did not contain a nontransforming avian tumor virus in amounts exceeding the titer of focus-forming virus. However, a low titered avian leukosis, preexisting in "normal" C/A cells could contribute to the envelope properties of fRO. This possibility is suggested by recent studies on rRO (15-16).

Host range of fRO. Among chicken cells only type C/A (line 7, Regional Poultry Laboratory, U.S.D.A., East Lansing, Michigan) was found regularly susceptible to fRO (Table I). However, titers of fRO in cultures derived from different individual C/A embryos varied. About two thirds of the em-

bryos yielded highly susceptible cultures. These were used as an arbitrary standard of susceptibility (relative efficiency of plating = 1). On one third of the C/A embryos titers of fRO were reduced from 5- to 20-fold. The same C/A embryos which were relatively resistant to fRO also showed a diminished susceptibility to rRO. High and reduced susceptibility of C/A cultures was a stable trait of the cells which persisted through several transfers.

C/O cultures could be divided into two categories: One was prevalent in a commercial line of White Leghorn embryos (Heisdorf and Nelson Farms, Redmond, Wash.) and was insusceptible to fRO. The other, represented by embryos of line 15I (Regional Poultry Laboratory, U.S.D.A., East Lansing, Mich.) showed a low but significant susceptibility to fRO. About 10% of the commercial C/O embryos also showed this low susceptibility. All C/O embryos, on which fRO induced foci, were also susceptible to rRO. Type C/B and C/AB chicken cells, known to be insusceptible to rRO were also uniformly resistant to fRO. Fibroblasts derived from Japanese quail or from ringneck pheasants were susceptible to fRO as they were to rRO. The host range of fRO is thus completely identical to that of rRO (6, 7, 14, 15).

Surface antigens of fRO. Several high-titered antisera produced in chickens against avian tumor virus subgroups A, B, C, and D failed to neutralize the infectivity of fRO. However, three sera prepared in ringneck pheasants against rRO also neutralized fRO. The titers of these sera against rRO and against fRO were identical. An antigenic relationship between fRO and rRO also was demonstrated by fluorescent antibody staining. All sera which specifically stained rRO-infected cells also reacted with fRO-infected cells.

Interference and facilitation. Table II summarizes the effects of avian leukosis viruses on the cellular susceptibility to fRO. Three types of responses were observed: (i) interference with fRO, (ii) unaltered cellular susceptibility, and (iii) facilitation of fRO infection. Interference with fRO was observed in C/A cultures preinfected with either subgroup B (RAV-2) or D (CZAV, RAV-50) avian leukosis viruses. This sensitivity to interference is identical to the one reported for rRO (7, 14). No significant change in cellular susceptibility to fRO was seen after preinfection of C/A cells with subgroup C leukosis viruses (RAV-7, RAV 49). Facilitation of infection with fRO was observed with C/O cells of line 15I. Whereas uninfected cultures of this type showed only a low susceptibility to fRO (Table I), preinfection of the cultures with subgroup A avian leukosis viruses brought the susceptibility up to the level of C/A cells (Table II). A similar facilitation on line 15I fibroblasts has been reported for rRO (18).

TABLE I. Host Range of fRO.

Cell type	No. of embryos tested	Relative efficiency of plating[a]
Chicken		
C/A	12	0.05 to 1.0
C/O[b]	21	$<10^{-3}$
C/O[c]	7	1.3 to 8 $\times 10^{-2}$
C/B	5	$<10^{-3}$
C/AB	3	$<10^{-3}$
Japanese quail	4	0.1 to 2.3
Ringneck pheasant	5	0.95 to 1.3

[a] The high plating efficiency found with most embryos of type C/A (Line 7, Regional Poultry Laboratory, U.S.D.A. East Lansing, Michigan) was arbitrarily set as 1.0.

[b] About 90% of the embryos from a White Leghorn line, Heisdorf and Nelson Farms, Redmond, Washington.

[c] All embryos of line 15I, Regional Poultry Laboratory, U.S.D.A. East Lansing, Michigan, and about 10% of the White Leghorn embryos, Heisdorf and Nelson Farms, Redmond, Washington.

Ability of fRO to reproduce in solitary infection. The reproduction of infectious rRO has been reported to be influenced by the type of host cell which is infected. After infection with rRO some cell types release only noninfectious particles whereas others release infectious rRO (15, 16). The synthesis of fRO is similarly affected by the cell (Table III). Single foci produced by fRO in

TABLE II. Effects of Avian Leukosis Viruses on Cellular Susceptibility to fRO.[a]

Preinfecting avian leukosis virus	Cell type	Relative efficiency of plating
RAV-2	C/A	1×10^{-3}
CZAV	C/A	$<10^{-2}$
RAV-50	C/A	3×10^{-3}
RAV-7	C/A	1.2
RAV-1	C/O, line 15I	17
RAV-5	C/O, line 15I	30 to 55

[a] Chick embryo fibroblasts were infected with the avian leukosis viruses listed in the table, transferred three times at 2-day intervals and challenged with fRO. Efficiencies of plating are expressed in relation to uninfected control cultures of the same cell type. Successful infection with each of the leukosis viruses was verified by demonstrating interference with a Rous sarcoma virus of the respective subgroup.

Japanese quail fibroblast cultures were free of infectious virus. In contrast, most single foci isolated from type C/A chicken cells contained infectious fRO. This host dependence of reproduction seen with fRO and rRO will be considered more thoroughly in another report (17).

Production of pseudotypes with fRO. Chick embryo fibroblast cultures infected and transformed by fRO were superinfected with avian leukosis viruses of subgroups A, B, C, and D. The virus present in the culture fluids 4 days after superinfection was harvested and characterized with respect to host range, sensitivity to interference and reaction with subgroup-specific, neutralizing antibodies. The majority of the focus-forming virus in these harvests consisted of pseudotype particles whose envelope properties were controlled by the avian leukosis virus which had been used to superinfect the culture. Only a small percentage of focus formers (from 0.2–2%) had retained the host range of fRO. These observations demonstrate that fRO can undergo phenotypic mixing with avian leukosis viruses to yield pseudotype virions.

Oncogenicity of fRO in ringneck pheasants. In order to test whether a virus which produces fusiform foci in tissue culture can also induce sarcomas in the animal, about 5 $\times$ 10^3 focus-forming units of fRO were injected into the wing web of 4-week-old ringneck pheasants. Three out of four animals developed sarcomas at the site of inoculation. Tissue sections were obtained from the tumors and stained with hematoxylin and eosin. A comparison with sections from sarcomas induced by rRO in the same species did not reveal conspicuous morphological differences of the tumor cells.

Discussion. Only one focus out of 110 was found to release fRO. The remainder produced fusiform RSV of the parental serotype. This was probably caused by the persistence of RAV in the majority of the foci picked, but further studies are necessary to confirm this assumption. Although no overt helper virus was detectable in stocks of fRO, the synthesis of infectious fRO progeny may nevertheless depend on some auxiliary genetic material which could preexist in the cell. The finding that infectious fRO is released from C/A but not from Japanese quail cells is in accordance with this suggestion. A similar dependence of reproduction on cell type has been found for rRO by Weiss (14) and by Hanafusa and co-workers (16). Weiss also observed that the cells capable of synthesizing infectious rRO contained the avian tumor virus group-specific antigen prior to infection. This antigen may indicate the presence of viral genetic material in the cells, although a complete virus has not been detected so far.

TABLE III. Virus Production in Foci Induced by fRO.[a]

Cell type	Number of embryos tested	Fraction of single foci-producing fRO
C/A	3	25/30
Japanese quail	7	0/48

[a] Chick or quail embryo fibroblast cultures were infected with a high dilution of fRO giving 10 foci or less per 35-mm petri dish. At day 7 foci were picked, and transferred singly into test tubes. The cells were destroyed by sonication and the presence of cell-free infectious virus in the focus was demonstrated by assay on quail fibroblasts.

The envelope-controlled properties of fRO and rRO were found to be identical. This plus the fact that both agents were derived from the Bryan high-titer strain of RSV makes it likely that they represent mutants of the same virus, differing in the gene which controls the shape of the transformed cell. Since fusiform and round foci produced in the same culture are easily distinguishable, the two mutants differing in this marker should prove useful in future genetic experiments.

Summary. A Rous sarcoma virus (RSV) is described which shares the envelope properties of RSV type O but causes infected cells to assume a fusiform shape. The agent induces sarcomas in susceptible birds. Japanese quail cells infected with a single particle of this virus fail to yield infectious progeny whereas chicken cells of type C/A readily reproduce the agent.

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