Nonproducing State of Rous Sarcoma Cells: Its Contagiousness in Chicken Cell Cultures

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Nonproducing Rous sarcoma cells of the chicken were capable of transmitting the Rous sarcoma virus genome to neighboring chick embryo fibroblasts. This transfer required close proximity of sarcoma and normal cells and may have been mediated by a subcellular infectious agent which was found to be released from nonproducing cells.

Solitary infection of chick embryo fibroblasts with the Bryan high-titer strain of Rous sarcoma virus (RSV) leads to the appearance of nonproducing (NP) Rous sarcoma cells. Such NP cells carry the genome of RSV and can be activated to release infectious RSV progeny by superinfection with an avian leukosis virus. This dependence of RSV maturation on another virus supports the conclusion that this strain of RSV is defective (11). NP cells are free of demonstrable amounts of viral envelope antigen, and RSV released from activated Rous sarcoma cells bears the envelope antigen of the helper virus (13, 30). This indicates that the defect of RSV lies in the production of the viral envelope, and that the helper virus activates RSV maturation by directing the synthesis of functional envelope material. However, although viral envelope antigen is missing from NP cells, an internal antigen of RSV is made in these cells (35).

Sarcomas which fail to release infectious virus also arise after infection of mammals by RSV (36). Again, the sarcoma cells contain the viral genome and an internal antigen of the virion (24, 14). However, their failure to produce viral progeny is probably not due to a defectiveness of the virus itself, since even strains of Rous sarcoma virus which are nondefective in the chicken (6-9) do not, as a rule, produce progeny in the mammal (1, 27). Rather, the absence of virus maturation appears to reflect an incompetence of the host cell. Mammalian Rous sarcoma cells are capable of transmitting the viral genome to neighboring chicken cells in the apparent absence of complete,

infectious virus. This transmission has been demonstrated numerous times in the animal host and in tissue culture (10, 16, 22, 23, 25, 26, 28, 29). It occurs with defective as well as nondefective strains of Rous sarcoma virus. The mechanism of this transfer of RSV from mammalian to avian cells is not fully understood.

In the course of extensive work with chicken NP cells, it was noted that, when a few NP cells were placed in a culture of normal fibroblasts, transformed cells often constituted the majority of the cell population after two to four transfers of the culture. The increase in the proportion of sarcoma cells appeared to proceed faster than could be expected from cell division alone. This observation suggested the possibility of a contact-mediated transfer of the RSV genome from NP to normal cells, similar to that seen in mixed cultures of mammalian Rous sarcoma cells and chicken fibroblasts. This possibility was explored in the experiments described in the present communication.

MATERIALS AND METHODS

Virus. Two pseudotypes (21) of the defective Bryan high-titer strain of RSV were used. One had as a helper Rous-associated virus type 1 (RAV-1), the other, Rous-associated virus type 2 (RAV-2). RSV(RAV-1) is a member of the avian tumor virus subgroup A, and RSV(RAV-2) belongs to subgroup B (15, 31-33). The helper viruses RAV-1 and RAV-2 were also used separately, as well as a subgroup B leukosis virus derived from the BAI-A strain of avian myeloblastosis and termed AMV-2. The preparation of virus stocks has been described previously (15). RSV was assayed according to the procedure of Rubin (19). Infectivity titers of noncytopathic leukosis viruses were determined with the fluorescent focus assay (30, 34) and are expressed in fluorescent focus-forming units. One

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fluorescent focus-forming unit corresponds to about 10 to 20 infectious units.

Chicken fibroblast cultures. Chick embryo fibroblasts were prepared from individual 10-day-old embryos according to published techniques (20). Part of the primary cultures from each embryo were tested by inoculation with RSV(RAV-1) and RSV(RAV-2) for genetic resistance to avian tumor viruses and by fluorescent-antibody staining for the presence of congenital leukosis virus infection. Congenitally infected cells were discarded. Genetic resistance to avian tumor viruses defined four cellular phenotypes (21): C/O cells were susceptible to avian tumor viruses of subgroups A and B, C/B cells were selectively resistant to viruses of subgroup B, C/A cells showed the reciprocal resistance directed against viruses of subgroup A, and C/AB cells were resistant to avian tumor viruses of both subgroups. Embryos of the C/O and C/B phenotype came from line 934-E of Hyline Farms and were obtained locally; C/A and C/AB embryos came from line 7 and line 7 $\times$ line 15 crosses of the Regional Poultry Laboratory of East Lansing, Mich. (4).

Isolation of NP Rous sarcoma cells. The procedures described by Hanafusa and co-workers (11) were used as modified for work previously reported (3). Supernatant samples of NP and of RSV-producing cultures were assayed for RSV after centrifugation for 15 min at 5,000 rev/min and after sonic treatment for 2 min to eliminate intact Rous sarcoma cells.

RESULTS

Genetically resistant NP cells: Activation with the excluded helper virus. NP Rous sarcoma cells of the C/A phenotype can be initiated by solitary infection with RSV(RAV-2). These C/A NP cells are genetically resistant to avian leukosis viruses of subgroup A, e.g., RAV-1. As a result, inoculation of C/A NP cultures with RAV-1 does not activate RSV maturation. In contrast, superinfection of such C/A NP cells with RAV-2 regularly leads to RSV production.

To test the limitations of the genetic resistance of C/A NP cells to RAV-1, the following experiment was carried out. Six single NP Rous sarcoma foci were picked from C/A cultures and placed in separate dishes with normal, unirradiated C/A cells as feeders. After two to three transfers at 3-day intervals, each of these NP cultures containing Rous sarcoma cells derived from a different single focus was divided into two parts (Fig. 1). One received normal C/B fibroblasts; the other was supplemented with normal C/A cells. At the next transfer, the cells of each culture were again divided, this time into three parts, and seeded in different dishes. At this point, cells from a single focus were present in two triplets of cultures, one triplet containing C/A feeders only, the other containing C/B as well as C/A feeder cells. These triplets were then treated as follows.

FIG. 1. Schematic representation of an experiment leading to activation of C/A NP cells with RAV-1. See Text for details.

One dish was inoculated with 10^6 fluorescent focus-forming units of RAV-1; a second, with 10^5 fluorescent focus-forming units of RAV-2; and the third remained uninfected (Fig. 1). The supernatant fluids of these cultures were harvested 3 days after RAV infection and were assayed for RSV in C/O, C/A, and C/B cells. This allowed subgroup typing of the progeny RSV. The results which were qualitatively the same for all six foci are summarized in Table 1. They show that the subgroup A leukosis virus, RAV-1, was capable of activating C/A NP cells when these cells were grown in the presence of the excluded virus, RAV-2, which as a member of subgroup B is not excluded from C/A cells, could activate RSV production in C/A NP cells regardless of the type of feeder cell present.

The activability of C/A NP cells by RAV-1 in the presence of C/B feeder cells could be explained in several ways: (i) The genetic resistance of C/A cells to RAV-1 was only quantitative, and the continuous presence of the excluded virus in large quantities supplied by the C/B cells led to an occasional successful infection of C/A NP cells with RAV-1. (ii) Viral genomes were transferred from cell to cell. The genome of RAV-1

TABLE 1. *Activation of RSV production in genetically resistant NP cells*

Composition of NP culture		Activating avian leukosis virus	RSV ^a in supernatant fluids of activated NP cultures assayed on		
NP cell type	Feeder cell type		C/O fibroblasts	C/A fibroblasts	C/B fibroblasts
C/A	C/A	None	0	0	0
C/A	C/A	RAV-1	0	0	0
C/A	C/A	RAV-2	+	+	0
C/A	C/A + C/B	None	0	0	0
C/A	C/A + C/B	RAV-1	+	0	+
C/A	C/A + C/B	RAV-2	+	+	0

^a Symbols: 0 = less than 10 focus-forming units of RSV per culture; + = 10² to 10⁶ focus-forming units of RSV per culture.

might thus have entered C/A NP cells, or the genome of defective RSV might have spread to C/B feeder cells making activation with RAV-1 possible. (iii) A genetic or physiological factor, determining sensitivity to subgroup A, may have been transferred from C/B feeder to C/A NP cells, again leading to activability by RAV-1. The next experiments will examine the first explanation.

Activation of RSV production in genetically resistant NP cells: Requirement for mixed culture with susceptible feeder cells. Different petri dishes containing 18 by 18 mm cover slips were seeded with (i) C/A NP cells and C/A normal fibroblasts, (ii) C/A NP cells and C/B as well as C/A normal fibroblasts, and (iii) normal C/B fibroblasts alone. After the cells had settled, the cover slips were rinsed gently with nutrient medium to remove cells which had failed to attach firmly to the glass. Pairs of cover slips were then glued with silicone grease to the bottom of new 100-mm petri dishes. The cover slips were placed as far apart from each other as the petri dish allowed, and were then covered with nutrient medium. The combinations of cover slips brought thus together in a given dish are listed in Table 2, columns 1 and 2. They included two in which both cover slips contained the same cell population, namely either C/A NP cells and C/A feeders, or C/A NP cells and C/B as well as C/A feeders. The third combination consisted of one cover slip carrying C/A NP and C/A feeders, and the other cover slip was seeded with normal C/B cells. All combinations were then inoculated with RAV-1. Infection was successful in the two combinations containing C/B cells, leading to the production of about 10⁵ fluorescent focus-forming units of progeny RAV-1 per culture by the 3rd day after

infection. No RAV-1 progeny was demonstrable in dishes containing only C/A cells. If the continued presence of RAV-1 was sufficient to overcome the genetic resistance of C/A NP cells, then both combinations containing RAV-1-releasing C/B cells should show RSV production. If, on the other hand, direct contact or close proximity between resistant C/A NP cells and RAV-1-producing feeders was required for activation of C/A NP cells by RAV-1, then those cultures in which the C/A NP cells and C/B fibroblasts were on separate cover slips should fail to show infectious RSV. The results of six such experiments are summarized in Table 2. Direct contact or close proximity of C/B feeders with C/A NP cells facilitated an efficient activation of RSV production by RAV-1. Separation of C/A NP cells from C/B feeders resulted only in an occasional, low level of RSV maturation, despite the fact that the RAV-1 concentration in the medium of all C/B cell-containing cultures was the same. Even this low level of RSV activation seen when C/B fibroblasts were separated from C/A NP cells may not have been due to successful infection of C/A NP cells by RAV-1. A rare cell could have become dislodged from one cover slip and settled on the other, thus creating contact between C/A and C/B cells. This possible cell migration was therefore excluded in another experiment.

Two cell combinations were seeded directly on 60-mm petri dishes. The first consisted of C/A NP cells and C/A feeder cells. The second included C/A NP cells and C/B as well as C/A feeder cells. Two dishes were prepared of each combination. One dish was left uninfected, and the other was inoculated with RAV-1. To provide a continuous supply of infectious RAV-1 in the supernatant fluid of those inoculated plates which contained only C/A cells, the medium of these dishes was replaced approximately every 6 hr with 5×10^6 fluorescent focus-forming units of RAV-1 in 5 ml of fresh culture fluid. Since the half-life time of RAV-1 is about 3 hr at 37 C (12), the medium changes prevented a drop of the available helper virus below a level of 10⁵ fluorescent focus-forming units per ml. The supply of RAV-1 in the inoculated cultures was kept up by 21 medium changes over a period of 5 days. During the same interval, those control cultures containing only C/A cells received the same number of virus-free medium changes. The cell combination including C/B feeder cells was inoculated only once with 10⁶ fluorescent focus-forming units of RAV-1 on the day the medium changes were started on pure C/A cultures. At the end of 5 days, the supernatant fluids of all dishes were

TABLE 2. *Effect of cell contacts on the activability of C/A NP cells by RAV-1*

Cell combinations (pairs of cover slips per dish) ^a		NP cells derived from focus no.	RSV(RAV-1) ^b
Cover slip no. 1	Cover slip no. 2		
C/A NP + C/A F	C/A NP + C/A F	1	0
		2	0
		3	0
		4	0
		5	0
		6	0
C/A NP + C/A F	C/B F	1	10
		2	15
		3	5
		4	2
		5	0
		6	5
C/A NP + C/A F + C/B F	C/A NP + C/A F + C/B F	1	1 × 10 ³
		2	5 × 10 ³
		3	2 × 10 ³
		4	2.5 × 10 ²
		5	1 × 10 ²
		6	5.5 × 10 ²

^a NP = nonproducing Rous sarcoma cells; F = normal chick embryo fibroblasts. A cover slip was occupied by about 1.6×10^5 cells. Mixed seedings contained equal proportions of normal and NP cells.

^b In culture medium on day 4 after addition of RAV-1. Expressed as focus-forming units per milliliter.

TABLE 3. *Prolonged exposure of C/A NP cells to RAV-1: failure to activate RSV synthesis*

Cell combination ^a	Helper virus	Assay of supernatant fluids ^b on		
		C/O fibroblasts	C/A fibroblasts	C/B fibroblasts
C/A NP + C/A F	None	0	0	0
C/A NP + C/A F + C/B F	None	0	0	0
C/A NP + C/A F	RAV-1 ^c	0	0	0
C/A NP + C/A F + C/B F	RAV-1	10 ³ to 2 × 10 ^{4d}	0	10 ³ to 2 × 10 ⁴

^a NP = nonproducing Rous sarcoma cells; F = normal chick fibroblast feeder cells. The various cell types were seeded in equal proportions. Total number of cells per dish was 1.2×10^6 .

^b Supernatant fluids were centrifuged at 5,000 rev/min for 15 min and were sonic treated for 2 min to eliminate intact Rous sarcoma cells. Samples were inoculated in amounts of 1 ml in the assay cultures listed in the table, and RSV foci were counted on the 7th day after inoculation. Titers are given in focus-forming units of RSV per milliliter.

^c Fresh RAV-1, 5×10^6 focus-forming units per culture, was supplied to the cells in approximately 6-hr intervals for 5 days.

^d Range of eight experiments.

harvested and assayed for RSV. If there was a small chance that RAV-1 could overcome the genetic resistance of C/A NP cells, then cultures containing only C/A cells should produce RSV after the 5-day exposure to the helper virus. If, however, contact or close proximity between C/B feeders and C/A NP cells was required for activation of the selectively resistant NP cells with RAV-1, only the cell combination including C/B feeders should produce RSV. The latter

expectation proved to be the correct one as demonstrated by the results summarized in Table 3. These results also show that the progeny RSV made in the presence of C/B cells belonged, according to its host range, to subgroup A as did the helper virus, RAV-1. The experiment represented by Table 3 was done eight times by use of C/A NP cells derived from different single foci. The results of all eight replicate experiments were qualitatively the same.

These observations indicate that, in the concentrations used (at least 10^5 fluorescent focus-forming units per ml for 5 days), RAV-1 cannot directly activate C/A NP cells. A close proximity of RAV-1-producing cells and C/A NP cells seems to be necessary for such an activation. This condition may facilitate an exchange of viral genetic material between NP cells and feeder cells. The transfer of the RSV genome from C/A NP cells to C/B feeders as well as the acquisition of the RAV-1 genome by C/A NP cells would result in activation of RSV production.

Transfer of the RSV genome from C/A NP cells to C/B feeder cells. Among the possible interactions of C/A NP cells and C/B feeder cells, the transmission of the RSV genome from NP to feeder cells appeared most interesting, because it could also contribute to the spread of malignant cellular properties within the animal host under natural conditions. Evidence for such a transfer of RSV is provided by the following experiments. C/A NP cells were grown together with C/B feeder cells in the same culture dish for periods varying from 6 to 12 days, with two to five transfers of the cultures. At the end of this period of co-cultivation, the cells were suspended with trypsin and plated in small numbers (from 50 to 500 NP and feeder cells) in 60-mm dishes containing 10^6 normal C/A fibroblasts. On the day after plating, the cultures were overlaid with nutrient agar. The feeder cells seeded onto the sheet of C/A fibroblasts were, of course, not distinguishable from the background of normal cells. However, the NP cells could be readily identified, because they were characteristically transformed and, in the course of 4 to 6 days, divided sufficiently to form discrete foci. Such foci were picked with capillary pipettes in the manner used for the isolation of NP cells (11, 30) and were placed singly in dishes containing normal C/A fibroblasts. If co-cultivation of C/A NP cells and C/B feeders had resulted in the transfer of the RSV genome to C/B feeder cells, some of the foci that appeared after plating small numbers of cells from mixed cultures should be of the C/B type. These should be activable only with an avian leukosis virus of subgroup A, e.g., RAV-1, whereas foci initiated by the original C/A NP cells should be activable only by an avian leukosis virus of subgroup B, e.g., RAV-2 or AMV-2. On the other hand, if mixed culture had not led to the acquisition of NP properties by some C/B feeder cells, then all foci derived from such cultures should retain the activation characteristics of the original C/A NP cells, and be susceptible only to helper viruses of subgroup B. This reasoning is summarized in Fig. 2.

The single foci isolated as described above

- (1) C/A NP CELL }
C/B FEEDER CELL } MIXED CULTURE
→ C/A NP CELL + C/B NP CELL
- (2) C/A NP CELL + RAV-1 → NO RSV
C/A NP CELL + RAV-2 → RSV(RAV-2)
- (3) C/B NP CELL + RAV-1 → RSV(RAV-1)
C/B NP CELL + RAV-2 → NO RSV

FIG. 2. Consequences of cultivating C/A NP and C/B feeder cells in the same container. C/B NP cells appear which are activable exclusively by subgroup A leukosis viruses (RAV-1). The original C/A NP can be activated only by a subgroup B leukosis virus (RAV-2).

FIG. 3. Cloning of Rous sarcoma cells after co-cultivation of C/A NP and C/B feeder cells. See text for details.

from mixed cultures of C/A NP cells and C/B feeders were therefore tested for activability with RAV-1 and RAV-2 or AMV-2. Each of the cultures implanted with a single focus was transferred twice and was divided each time, and, of the resulting four dishes, one was left uninfected and the other three were separately infected with 10^5 focus-forming units of RAV-1, RAV-2, and AMV-2, respectively. Figure 3 gives a schematic representation of these experimental procedures. After 3 to 5 days, the supernatant fluids of the

TABLE 4. *Activability of single Rous sarcoma foci obtained from mixed cultures of C/A NP and C/B feeder cells^a*

Determination	No. of foci
Total no. of single foci isolated and tested.....	106
Activable with RAV-1 alone.....	50
Activable with RAV-2 or AMV-2....	6
Activable with RAV-1 as well as RAV-2 or AMV-2.....	16
Not activable.....	34

^a Single foci from mixed populations of C/A NP and C/B feeder cells were obtained by seeding the co-cultivated cells on C/A fibroblasts. The resultant foci of Rous sarcoma cells were picked and transferred onto new C/A fibroblasts. After two further transfers, samples of these cultures were activated with various avian leukosis viruses (Fig. 3).

cultures were harvested and assayed for RSV. The results, compiled in Table 4, indicate that during co-cultivation of C/A NP cells with C/B feeder cells the latter acquired the RSV genome. Cloning of such a culture yielded NP foci of the C/B as well as of the C/A type, as could be demonstrated by isolating such foci and activating RSV maturation with avian leukosis viruses of subgroups A and B. In the majority of the activable foci, only RAV-1 was effective in aiding the maturation of RSV (Table 4). These foci were therefore of the C/B type. A smaller proportion responded to RAV-2 or AMV-2 only and was classified as belonging to the original C/A type. RSV produced with the aid of RAV-1 was a member of subgroup A and formed foci only on C/O and C/B fibroblasts. RSV produced after activation with RAV-2 belonged to subgroup B and transformed only on C/O and C/A fibroblasts. The finding of foci activable by subgroup A as well as B avian leukosis viruses (Table 4) appeared at first surprising. However, such foci may have arisen from incomplete dissociation of cells in the first cloning step (Fig. 3), and may have been descendants of more than one cell, including the C/A and C/B types. Doubly activable NP cultures could also result from the back transfer of the RSV genome from C/B NP cells to C/A feeder cells (Fig. 3, step 3) or from somatic hybridization of C/A and C/B cells. The controls which did not receive an avian leukosis virus failed to produce detectable quantities of infectious RSV. A sizable proportion of focus isolations (Fig. 3, step 2) failed to yield cultures in which RSV maturation was activable with either subgroup A or B leukosis viruses after two transfers. Microscopic inspection at the time of

TABLE 5. *Activability of single Rous sarcoma foci obtained from mixed cultures of C/A NP and C/B feeder cells^a*

Determination	No. of foci
Total no. of single foci isolated and tested.....	61
Activable with RAV-1 alone.....	8
Activable with RAV-2 or AMV-2....	0
Activable with RAV-1 as well as RAV-2 or AMV-2.....	5
Not activable.....	48

^a Single foci from mixed populations of C/A NP and C/B feeder cells were obtained by seeding the co-cultivated cells on C/AB fibroblasts. The resultant foci were picked and transferred onto new C/AB fibroblasts. After two further transfers, samples of these cultures were activated with various avian leukosis viruses (Fig. 3).

activation showed such cultures free from transformed cells. This apparent loss of NP cells during transfers of cultures was even more prominent when C/AB cells were used as feeders for the cloning and replating of NP cells (Fig. 3, step 2). Under these conditions, the majority of the NP foci did not persist until superinfection with a leukosis virus (Table 5). This curious finding is being investigated further. The data of Table 5 also demonstrate, like those of Table 4, that, during co-cultivation of C/A NP and C/B feeder cells, a transfer of the RSV genome to the feeder cells took place and resulted in NP cells which were activable by RAV-1 only. The doubly activable foci listed in Table 5 may again be tentatively explained by an incomplete dispersion of cells during cloning (Fig. 3, step 1) or by somatic hybridization. These possibilities need further study.

Observations on a subcellular transforming principle from NP cultures. Several hundred samples of supernatant fluid from NP cultures were tested for viral infectivity in the course of the present studies. The great majority of these samples showed no focus-forming activity (< 1 focus-forming unit per ml). The control values in Tables 1 to 5 attest to this. However, a few supernatant fluids from NP cultures were found which on occasion produced small numbers of RSV foci when inoculated in chick fibroblasts. Titers ranged between approximately 2 and 35 focus-forming units per ml medium from NP cultures with 10^5 to 5×10^5 transformed cells. At first, this focus-forming activity appeared to be due to a few intact NP Rous sarcoma cells which had been inadvertently harvested with the supernatant medium and had somehow not been

TABLE 6. *Focus formation by filtered^a supernatant fluid of NP cultures*

Supernatant fluid of NP culture no.	Assay ^b on	
	C/O type cells	C/A type cells
1	1	3
2	0	4
3	3	25
4	2	18
5	1	4
6	0	3
7	4	35
8	0	2
9 ^c	525	540

^a Membrane filter, 0.45 μ average pore diameter.

^b Number of foci per plate inoculated with 1 ml of supernatant fluid. Figures are from a representative experiment with susceptible embryos.

^c Control: RSV (RAV-2), 0.1 ml, 1:10.

eliminated by the routine centrifugation and sonic treatment. This was indicated by the finding that focus-forming activity in NP samples could often not be demonstrated after storage at -80°C . Freezing and thawing impairs the viability of whole cells, but leaves complete RSV relatively unharmed. The foci formed by supernatant fluids of NP cultures were themselves nonproducing. Cultures on which such foci appeared were passaged for several weeks, showing in their medium at most the unstable, low-titered focus-forming activity which had been noted in the original NP culture. However, large amounts of RSV were released after superinfection with an avian leukosis helper virus.

To eliminate this presumably cellular focus-forming activity detected in the medium from NP cultures, several positive samples were passed through a membrane filter (Millipore Corp., Bedford, Mass.), with an average pore diameter of 0.45 μ . Focus-forming activity was still present in the filtrate. This strongly suggested that the transforming principle was subcellular. Further support for this suggestion came from the observation that supernatant samples of NP cultures induced focus formation only in chick fibroblasts derived from certain embryos. Highly susceptible embryos were of the C/A phenotype, but not all C/A embryos showed this susceptibility. Occasionally, foci were seen on C/O cultures inoculated with supernatant medium from NP cells (Table 6). C/B type embryos have not yet been studied in sufficient number to allow an assessment of their susceptibility. If the transforming principle from NP cultures consisted of intact, replicating Rous sarcoma cells, the number of foci

should be the same on all types of assay cultures. Since this is not the case, it is concluded that NP cells release a subcellular agent which is capable of transforming certain types of chicken cells.

DISCUSSION

In mixed cultures of C/A NP and C/B feeder cells, a transfer of the RSV genome from NP to normal cells takes place. This facilitates activation of RSV maturation by RAV-1, an avian leukosis virus excluded from C/A cells but capable of infecting C/B cells. The mechanism of this viral spread in NP cultures remains unknown. The requirement for close proximity of, or contact between, the interacting cells favors a direct transfer of the RSV genome, perhaps through cytoplasmic bridges or by cell fusion. The subcellular, focus-forming agent recovered from NP cultures may, however, contribute to the dissemination of RSV in the experimental system studied, if it is assumed that this agent preferentially infects the immediate neighbors of the NP cell from which it is released.

The finding of infectivity in NP cultures is probably related to the small number of morphologically complete viral particles which can be seen with the electron microscope on the surface of NP cells (3, 5; Haguenu and Hanafusa, *personal communication*). These observations suggest that infection with defective RSV can result in the formation of some progeny virus. The defect of the Bryan high-titer strain appears therefore not to be absolute. Nevertheless, the amount of progeny RSV released by NP cells is small, and this virus has a very restricted host range. Superinfection of NP cells with an avian leukosis helper virus is still needed to obtain high titers of RSV. Such activation results in the helper control of the RSV envelope (13, 30).

It is conceivable that the infectivity discovered in NP cultures is due to accidental infection with a new helper virus. Experiments designed to test this possibility and to compare the agent from NP cells with the common, helper-dependent RSV are now in progress.

The spread of the RSV genome from mammalian Rous sarcoma cells to adjacent chicken cells may also include an extracellular state of the virus. Avian tumor virus-like particles have indeed been found in some mammalian Rous sarcomas (18), and even infectious virus has been demonstrated in a few instances (2, 17).

The present paper has concentrated on the transfer of the RSV genome from NP to feeder cells. A contact-dependent transfer of RAV-1 from C/B feeder cells to C/A NP cells has been suggested as a possibility and is now under study.

It is also still undecided whether C/A NP cells can acquire a genetic or physiological factor from C/B cells which endows the resistant NP cells with susceptibility to avian tumor viruses of sub-group A.

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