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NOTES

Further Evidence for the Existence of a Viral Envelope Protein Defect in the Bryan High-Titer Strain of Rous Sarcoma Virus

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Electron microscopy observations of purified Bryan high-titer Rous sarcoma virus (BH RSV) using the freeze-drying technique showed that progeny made in the absence of a helper virus lacked visible surface projections or spikes. Phenotypic mixing experiments employing BH RSV and a thermolabile mutant of vesicular stomatitis virus, *tl* 17, yielded no evidence of pseudotype formation. Since *tl* 17 is known to be defective for an envelope glycoprotein, the lack of successful phenotypic mixing with BH RSV is consistent with the observed absence of viral spikes.

The isolation of "nonproducer" transformed cells after infection of chicken embryo cells with the Bryan high-titer strain of Rous sarcoma virus (BH RSV), the subsequent finding that production of infectious sarcoma virus could be rescued from such cells by superinfection with helper avian leukosis viruses, and finally the demonstration that the host range and type specificity of the resulting rescued sarcoma virus depended entirely on the helper virus used led to the hypothesis that BH RSV was a defective virus unable to make functional viral envelope proteins (5, 6). This hypothesis is borne out by analysis of the structural proteins of BH RSV (−) which has shown that, in fact, the major viral envelope glycoproteins, gp85 and gp37, are either absent or modified in their properties (12).

The experiments to be described in this communication present further evidence consistent with this hypothesis but which have been obtained from quite different technical approaches.

Cell lines obtained from single transformed cell colonies produced in soft agar suspension by BH RSV-infected Japanese quail embryo cells have been in culture since November 1971 (3). In particular, for these investigations a cell line designated BH RSV(−)Q clone 3, a nonproducer, and a cell line designated BH RSV (*chf*)Q clone 4, a producer of infectious virus at high titers, have been used. BH RSV(*chf*)Q clone 4 inadvertently became infected with *chf* (7), the helper virus used in preparation of the original BH RSV stock, simultaneously with the initial BH RSV infection. That BH RSV(−)Q clone 3 is indeed infected with BH RSV has been demonstrated in the following ways: (i) production of sarcoma virus may be readily rescued from these cells by inoculation of as little as one infectious interfering unit of avian leukosis virus; (ii) noninfectious BH RSV(−) from these cells has been shown to be capable of establishing infection and transformation if introduced into cells by Sendai virus-mediated fusion (Young C. Chen, personal communication); and (iii) noninfectious BH RSV(−) from this cell line has been radioactively labeled and analyzed for its constituent viral structural proteins (M. S. Halpern, personal communication).

For the electron microscopy experiments to be described, virus was purified from medium supernatants harvested every 12 h. Purification was accomplished without pelleting the virus; rather, centrifugation into a cushion of 55% sucrose, followed by equilibrium density gradient centrifugation through a gradient of 20 to 55% sucrose, was used. The visible virus band was recovered by puncturing the bottom of the tube and collecting fractions. After adding a hydrophilic agent, Bacitracin (4), the virus in these bands was adsorbed onto electron microscope grids bearing a carbon-coated Pioloform membrane. The grids were rinsed with phosphate-buffered saline (pH 7.2), contrasted with a 2% phosphotungstic acid (pH 7.2) solution, and subjected to freeze-drying (10) using Balzer's freeze-etching apparatus.

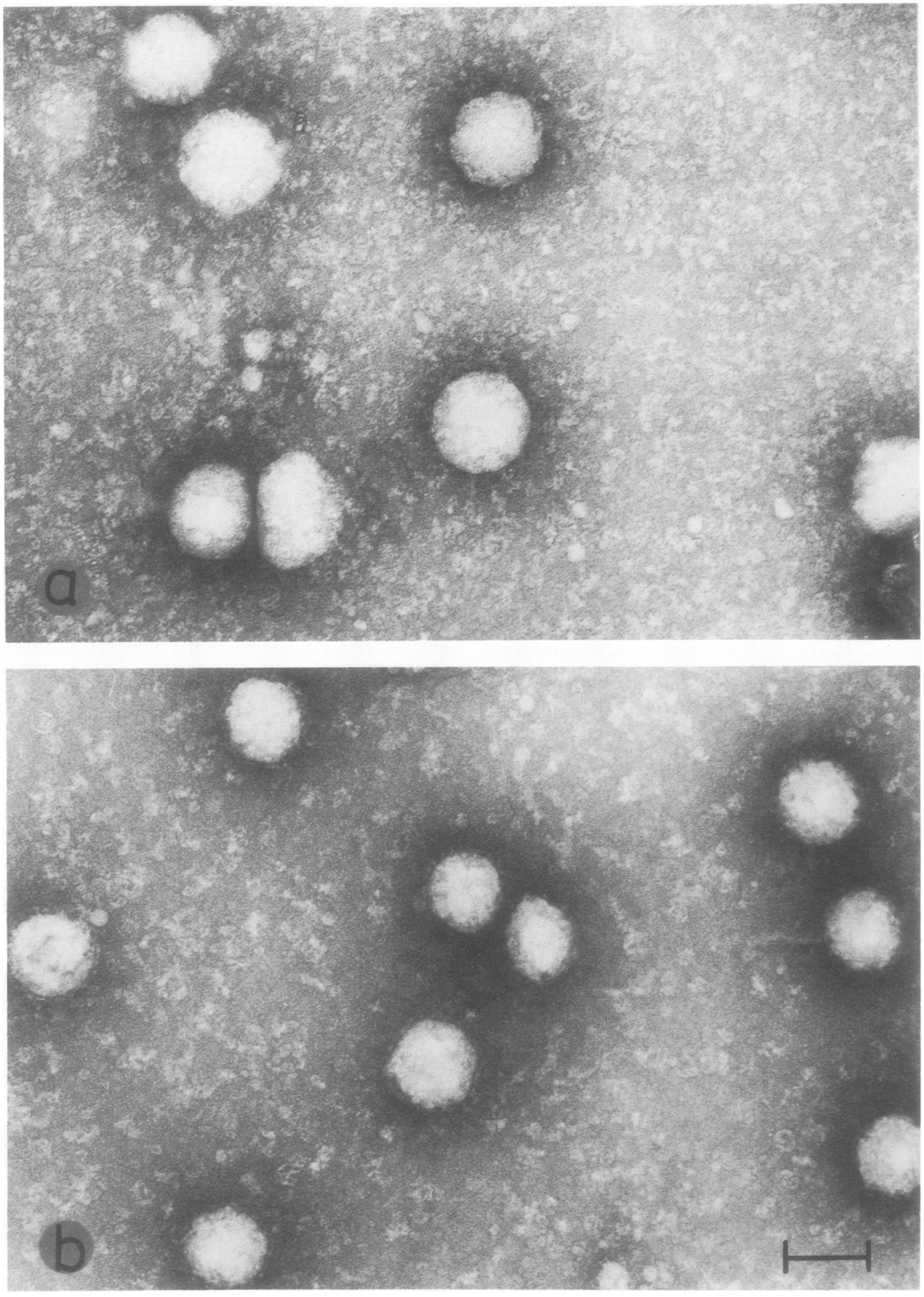

FIG. 1. (a) *Freeze-dried BH RSV(−) after contrasting with 2% phosphotungstic acid. Note the lack of spikes on the surface of virus particles.* (b) *Freeze-dried BH RSV(chf) prepared as for Fig. 1a. Note the presence of spikes on the surface of virus particles. Bar represents 100 nm.*

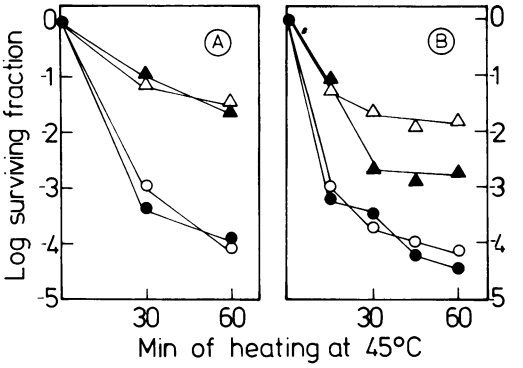

FIG. 2. *Thermal inactivation curves of VSV mutant t1 17 grown in normal Japanese quail cells, in Rous-associated virus type 3-infected Japanese quail cells, in BH RSV(chf)Q clone 4, and in BH RSV(−)Q clone 3 cell lines. Infectious tissue culture fluids were diluted 1:10 with phosphate-buffered saline (pH 7.2) containing 5% calf serum and heated for intervals at 45.0 ± 0.1 C. Surviving virus infectivity was determined by plaque assay in Japanese quail cells (A) and in C/O phenotype chicken embryo cells (B). Note that the pseudotype formation with BH RSV(chf)Q clone 4 cells was most apparent when assays were performed on quail cells, since the susceptibility of these cells to subgroup E is approximately 10-fold higher. Symbols: ●, BH RSV(−)Q clone 3; ○, Japanese quail cells; ▲, BH RSV(chf)Q clone 4; △, Japanese quail cells infected with RAV-3.*

The freeze-drying procedure preserves original viral morphology and has allowed visualization of viral surface projections even with some viruses which failed to show such projections after conventional negative staining (10). The application of this technique revealed that BH RSV(−) lacked surface projections (Fig. 1a) whereas BH RSV(*chf*) displayed the characteristic knobbed spikes of the avian RNA tumor viruses (Fig. 1b). These spikes are known to be composed of the two glycoproteins, gp85 and gp37 (1, 11), and serve as determinants for the host range and type specificity of these viruses (14). Hence, the ultrastructural characteristics of BH RSV(−) and BH RSV(*chf*) as observed in Fig. 1a and b conform to what might be expected from their known biological and biochemical properties. BH RSV(−) clearly lacks at least the greater part of each surface spike, but the background caused by the crystalization of the contrasting material makes it impossible to conclude absolutely that no surface structures remain associated with these virus particles. It is possible that the gp37 or another protein might be present.

It has been shown that the vesicular stomatitis virus (VSV) thermolabile glycoprotein-defective mutant VSV *t1* 17 can be phenotypically mixed with RNA tumor viruses (9, 15–17; R. A. Weiss, D. Boettiger, and D. N. Love, Cold Spring Harber Symp. Quant. Biol., in press) to produce VSV progeny which contain the RNA tumor virus envelope glycoproteins and which are accordingly much less sensitive to inactivation at 45 C than the parent. This mutant was generously supplied to us by J. Zàvada and has been employed in further experiments with BH RSV(−). For phenotypic mixing experiments, VSV *tl* 17 was grown at the permissive temperature (31 C) in both BH RSV-transformed cell lines, in normal Japanese quail embryo cells, and in Japanese quail embryo cells infected with an avian leukosis virus, Rous-associated virus type 3, subgroup A. Samples from these mixed infections were harvested when cytopathogenic effects were observed, at about 20 h after infection. The termolability at 45 C was measured in inactivation kinetic experiments which were assayed with a plaque formation test performed on Japanese quail embryo cells and C/O phenotype chicken embryo cells.

VSV *tl* 17 grown in BH RSV(−)Q clone 3 or in normal Japanese quail embryo cells was thermolabile in heat-inactivation tests (Fig. 2), whereas this mutant grown either in BH RSV(*chf*)Q clone 4 or in Rous-associated virus type 3-infected Japanese quail embryo cells became relatively resistant to heat inactivation, hence had undergone phenotypic mixing with avian tumor viral envelope glycoproteins. That this was indeed the case could be shown by the fact that the heat-resistant fraction in the phenotypically mixed virus could be quantitatively neutralized with antisera specific for the avian tumor virus used. The possibility that genetic reversion had occured in the VSV mixed infection stocks found to be heat resistant was excluded by showing that the progeny of this virus was exclusively thermolabile after one growth cycle in normal Japanese quail cells at the permissive temperature.

From the absence of phenotypic mixing between BH RSV(−) and VSV *tl* 17, it could be concluded that no functional viral envelope glycoprotein was present in the infected cell line. Studies of the apparent size of the BH RSV(−) genome (2, 13) have suggested that the information content is smaller than that of the helper-independent avian sarcoma viruses. Furthermore, M. S. Halpern (personal communication) has demonstrated that the gp85 glycoprotein is totally absent, not only from virus particles but also from BH RSV(−)Q clone 3 cell lysates. It therefore seems quite probable, as suggested by Kawai and Hanafusa (8), that BH RSV(−) is indeed a natural deletion mutant

lacking precisely the genetic information for gp85.

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