JOURNAL OF VIROLOGY, Nov. 1973, p. 1184-1186

Vol. 12, No. 5
Printed in U.S.A.

Antigenic and Biochemical Characterization of the C-type Particle of the Stable Porcine Kidney Cell Line PK-15

W. A. WOODS, T. S. PAPAS, H. HIRUMI, AND M. A. CHIRIGOS

National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014, and Boyce Thompson Institute, Yonkers, New York 10701

Received for publication 6 March 1973

The C-type particles observed by electron microscopy in PK-15 cells were demonstrated to have biochemical and biophysical properties associated with the oncornavirus group: density of 1:16 in a sucrose gradient, 70*S* RNA, and the RNA-dependent DNA polymerase. The group-specific interspecies antigen, gs-3, was not present. Evidence of a latent infection with a porcine parvovirus was also obtained.

Armstrong et al. (1) and Breese (3) have described the occurrence of C-type viral particles in stable pig kidney cell lines; the report of Howard et al. (6) describing "C"-type particles in lymphosarcomas induced in ^{90}Sr-fed mini-pigs apparently adds another mammalian species to the list of animals naturally infected with C-type viruses associated with tumors of lymphoid tissues and in "transformed" normal cell lines. However, the inclusion of such latent viruses in the oncornavirus group could only be tentative awaiting demonstration of biochemical properties associated with this group of agents.

We report here biochemical and biophysical properties of the C-type particles chronically infecting the stable porcine kidney cell line PK-15, as well as antigenic evidence for the presence of a latent pig parvovirus infection of these cells.

PK-15 cells grown in Lab-Tek chamber slides (Lab-Tek Products, Westmont, Ill.) were examined for type- and group-specific antigens by immunofluorescent staining (11). Specific cytoplasmic fluorescence was demonstrated in PK-15 cells specifically stained (Fig. 1A) with antibody to the PK-15 virus-like particles raised in a weanling mini-pig by T. Kawakami, University of California, Davis, Calif. Uninfected secondary embryonic pig kidney cell cultures or murine cells chronically infected with Moloney sarcoma virus were not stained by the immune or normal pig sera. Sera containing high titer antibody to murine or feline gs-1 or the gs-3 interspecies antigen did not stain the PK-15 cells, indicating a lack of these oncornavirus group-specific antigens. Nuclei of approximately 5 to 10% of the PK-15 cells were stained with both normal and anti-PK-15 virus sera from the same animal. Since this normal serum contained hemagglutinin inhibition antibody titer of 1:320 against porcine parvovirus, with no evidence of antibody to other common porcine viruses, the staining is suggestive of a latent parvovirus infection.

Many extracellular virus-like particles (Fig. 1B) were observed in PK-15 cells by electron microscopy (5, 7, 9). Intracisternal particles were occasionally seen in some cells (Fig. 1C). Particles budding from the surface of the host cells were frequently observed. The morphology of the particles was similar to that of "type C" particles previously described by others (1, 2, 4). No particles resembling any other known viruses were seen in either the cytoplasm or nucleus of the host cells.

Virions labeled with ^{3}H-uridine were concentrated (8) and purified by density gradient centrifugation on a 15 to 60% (wt/wt) linear sucrose gradient. A sharp incorporation peak at a density of 1.17 g/ml (Fig. 2A) was regularly obtained. Nucleic acid extracted from the radioactive ρ = 1.17 g/ml band was shown to be 70*S* (Fig. 2B).

The same fraction derived from unlabeled cultures demonstrated polymerase activity (10), which was dependent on exogenous template (Table 1).

When pancreatic RNase was included in the incubation mixture, the endogenous activity

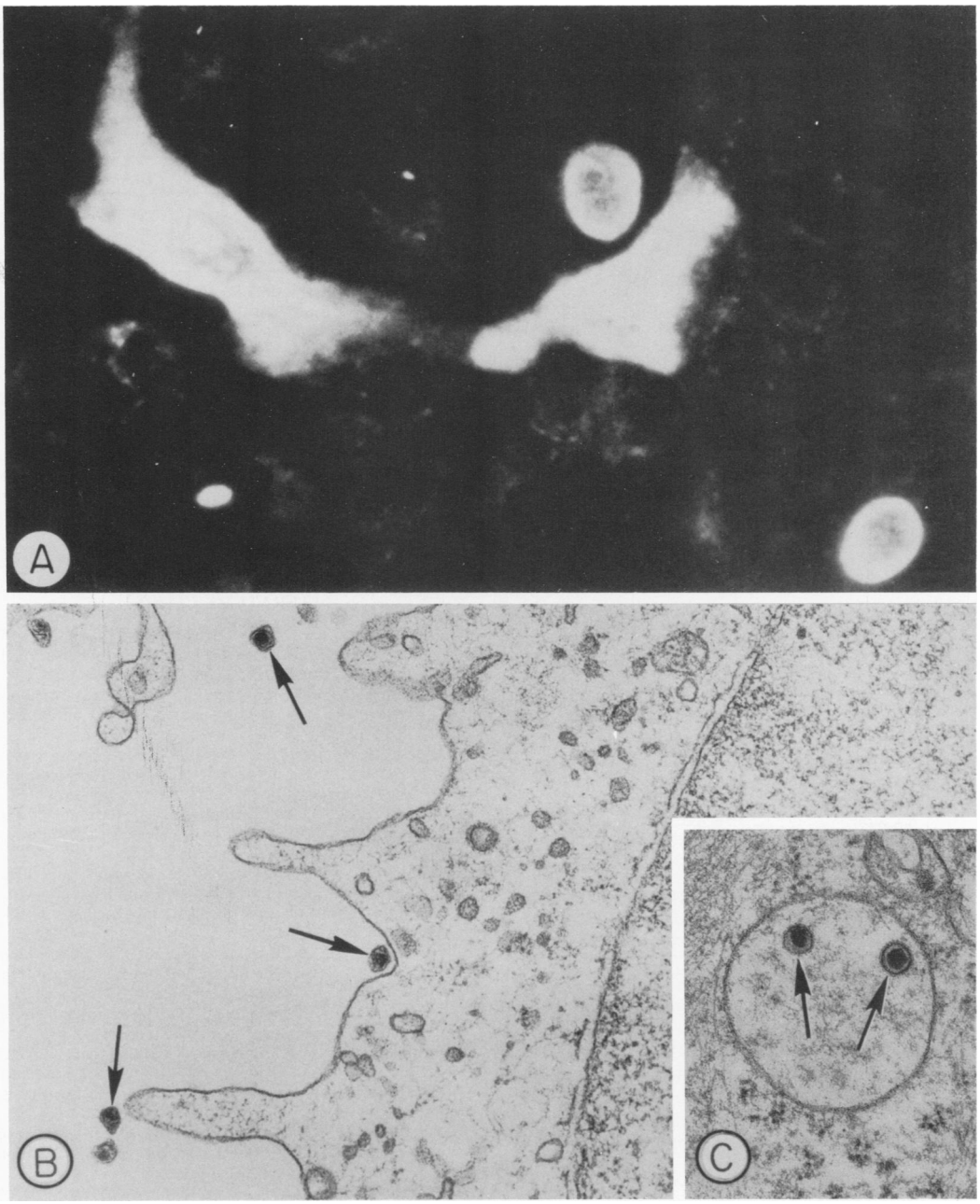

FIG. 1. *A, Fluorescent antibody staining of PK-15 cells. Seven-day culture stained with porcine anti-PK-15 particle serum and rabbit anti-porcine gamma globulin conjugate. ×1200. B, Extracellular C-type particles (arrows) in the pig kidney cell (PK-15) cultures at the sixth day of subcultivation. ×37,800. C, Intracisternal C-type particles (arrows) in the same culture. ×41,800.*

was markedly reduced, indicating that the endogenous activity depends on an RNA template. When the endogenous reaction was measured, omission of a single deoxynucleotide triphosphate markedly reduced DNA synthesis, indicating polymerization was observed and not simply terminal addition of triphosphate.

The conditions employed for disrupting PK-15 C-type particles and assaying its polymerase are optimal for the RLV but not necessar-

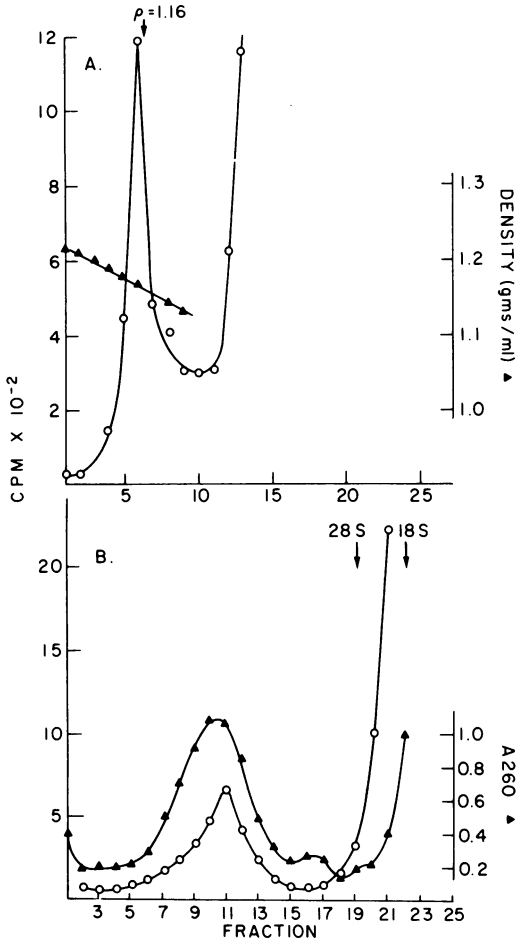

FIG. 2. *A, Sedimentation profile of ^{3}H-uridine-labeled PK-15 C-type particles. PK-15 cell extract labeled with ^{3}H-uridine was layered on a 15 to 60% sucrose gradient as described in Materials and Methods. Sedimentation is from right to left. B, Sedimentation profile of RNA extracted from ^{3}H-uridine-labeled PK-15 C-type particle* (O——O), *and avian myeloblastosis virus (AMV)* (Δ——Δ), *0.6 g wet weight. AMV (estimated by ATPase) was mixed with ^{3}H-uridine-labeled PK-15 virions on (5 to 30% [wt/vol]) sucrose gradients as described in Materials and Methods. Sedimentation is from right to left.*

ily for PK-15 particles. This perhaps can explain the 100-fold less polymerase activity in PK-15 particles than the RLV. Alternatively, the RLV virus was a highly purified preparation free of contaminants, whereas the PK-15 particle preparation had been subjected to only one sucrose gradient banding.

TABLE 1. *Reverse transcriptase activity of PK-15 C-type particles*[a]

Template	^{3}H-dTTP incorporated (pmole/h)		
	PK-15 C-type particles from cells	PK-15 C-type particles from supernatant fluid	RLV
Endogenous	6.7	7.20	3.7
Endogenous + RNase	0.2	0.25	
poly(rA)·(dT)$_{10}$	18.8	21.00	10.5

[a] Assays were as described in Materials and Methods. The reaction mixture was 25 μliters; amount of protein used for these assays was 45 μg of PK-15 C-type particle from cells, 55.5 μg of PK-15 from supernatant fluid, and 0.29 μg of RLV.

This investigation was conducted in part under Public Health Service contract NIH-NCI-E-73-3205, under the Special Virus Cancer Program of the National Cancer Institute.

We thank Kazuko Hirumi of Boyce Thompson Institute and Judith Massicot, Joseph Webb, and Thomas Pry of National Cancer Institute for their capable technical assistance.

LITERATURE CITED

1. Armstrong, J. S., J. S. Porterfield, and A. T. de Madrid. 1971. C-type virus particles in pig kidney cell lines. J. Gen. Virol. **10**:195-198.
2. Bernhard, W. 1960. The detection and study of tumor viruses with the electron microscope. Cancer Res. **20**:712-727.
3. Breese, S. S., Jr. 1970. Virus-like particles occurring in cultures of stable pig kidney cell lines. Arch. Gesamte Virusforsch. **30**:401-404.
4. Dalton, A. J. 1972. RNA tumor viruses—terminology and ultrastructural aspects of virion morphology and replication. J. Nat. Cancer Inst. **49**:323-327.
5. Hirumi, H., G. J. Burton, and K. Maramorosch. 1971. Electron microscopy of Friend murine leukemia virus in the mid-gut of experimentally infected mosquitoes. J. Virol. **8**:801-804.
6. Howard, E. B., W. J. Clark, and P. L. Hackett. 1968. Experimental myeloproliferative and lymphoproliferative diseases of swine. Bibl. Haematol. **31**:255-262.
7. Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**:409-414.
8. Robinson, H. L. 1967. Isolation of noninfectious particles containing Rous sarcoma virus RNA from the medium of Rous sarcoma virus-transformed nonproducer cells. Proc. Nat. Acad. Sci. U.S.A. **57**:1655-1662.
9. Venable, J. H., and R. Coggeshall. 1965. A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. **25**:407-408.
10. Wilson, S. H., and E. L. Kuff. 1972. A novel DNA polymerase activity found in association with intracisternal A-type particles. Proc. Nat. Acad. Sci. U.S.A. **69**:1531-1536.
11. Woods, W. A., J. Massicot, and M. A. Chirigos. 1970. An immunofluorescent focus assay for Gross leukemia virus. Proc. Soc. Exp. Biol. Med. **135**:772-777.