

Prevalence of Type R Virus-like Particles in Clones of BHK-21 Cells

The BHK-21 line of Syrian hamster fibroblasts (1) is used widely for research on viruses and on the neoplastic transformation of cells *in vitro*. Bernhard and Tournier (2) were the first to describe a virus-like particle in BHK-21 clone 13 (BHK-21/13) cells. Subsequent studies have confirmed the presence of this particle in BHK-21/C13/TC6/A (3), BHK-21F (4), and BHK-21/13S (5). A detailed study of the morphogenesis of this virus-like particle was reported by Thomas and his associates (6). Despite the detection of such particles in several clones of BHK-21 cells, and cells derived from spontaneous or induced hamster tumors (2, 7, 8), the widespread occurrence of this virus-like particle has received only limited recognition. The virus-like particle in question is distinguished by the presence of characteristic electron-dense radial structures which appear to emanate from the nucleoid. The radial structure of the particle differentiates it from the Bernhard type C virus particle characteristic of the murine leukemia viruses. To avoid confusion we shall refer to the hamster virus-like particle as a type R (radial) virus-like particle. It is

the purpose of this communication to document the presence and distribution of the type R virus-like particle in all clones of BHK-21 studied to date and to alert investigators to the presence of this particle.

The clones of BHK-21 cells selected for this study are listed in Table 1. The origins of these clones of BHK-21 and other clones known to contain type R virus-like particles have been previously described in the literature (9-12). Two other established hamster cell lines, HaK and RPMI-1846 (13), and normal and virus-transformed hamster cells were also examined. All hamster cells with the exception of RPMI-1846 cells were grown as monolayers in Eagle basal medium (Earle salts) supplemented with 15% fetal bovine serum. RPMI-1846 cells were propagated in McCoy 5A medium (modified) containing 20% fetal bovine serum. Incubation was carried out at 37° in an atmosphere of 4% CO₂-96% air.

Tissues or tissue culture cells for electron microscopy were fixed in 2.5% phosphate-buffered glutaraldehyde, postfixed in veronal-acetate buffered 1% OsO₄ and embedded in Epon 812. Thin sections were cut with a Porter-Blum MT-1 microtome and stained with a saturated aqueous solution of uranyl acetate and lead citrate prior to ex-

TABLE 1
OCCURRENCE OF TYPE R VIRUS-LIKE PARTICLES IN HAMSTER CELLS OF DIVERSE ORIGINS

Cell type	Relative concentration of type R particles	Source of cell
Established hamster cell lines		
BHK-21/4	++++	Parke-Davis and Co. (see Ref. 12)
BHK-21/13	++	American Type Culture Collection
BHK-21/13	++	Industrial Biological Laboratories
HaK	-	American Type Culture Collection
RPMI-1846	-	American Type Culture Collection
Virus-induced hamster tumor		
SV-40 induced fibrosarcoma	+	Dr. F. E. Payne, The University of Michigan
Normal hamster tissue or cells		
Embryonic hamster kidney cells	-	Dr. G. Anderson, Michigan State Department of Health
Germinal Centers from hamster Peyer's patches	-	The University of Michigan

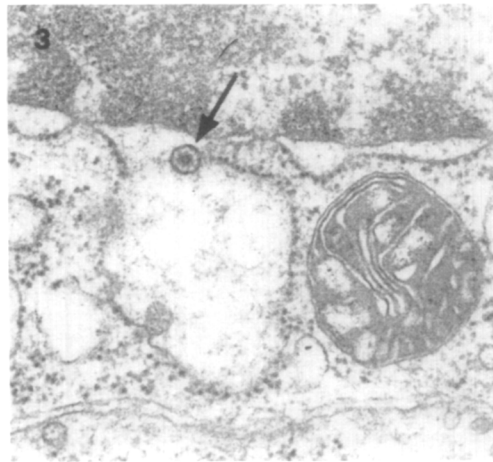
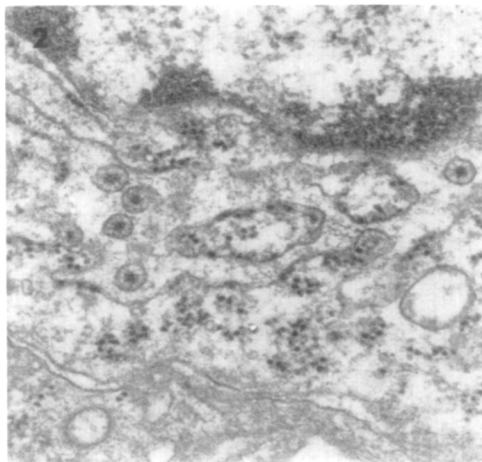
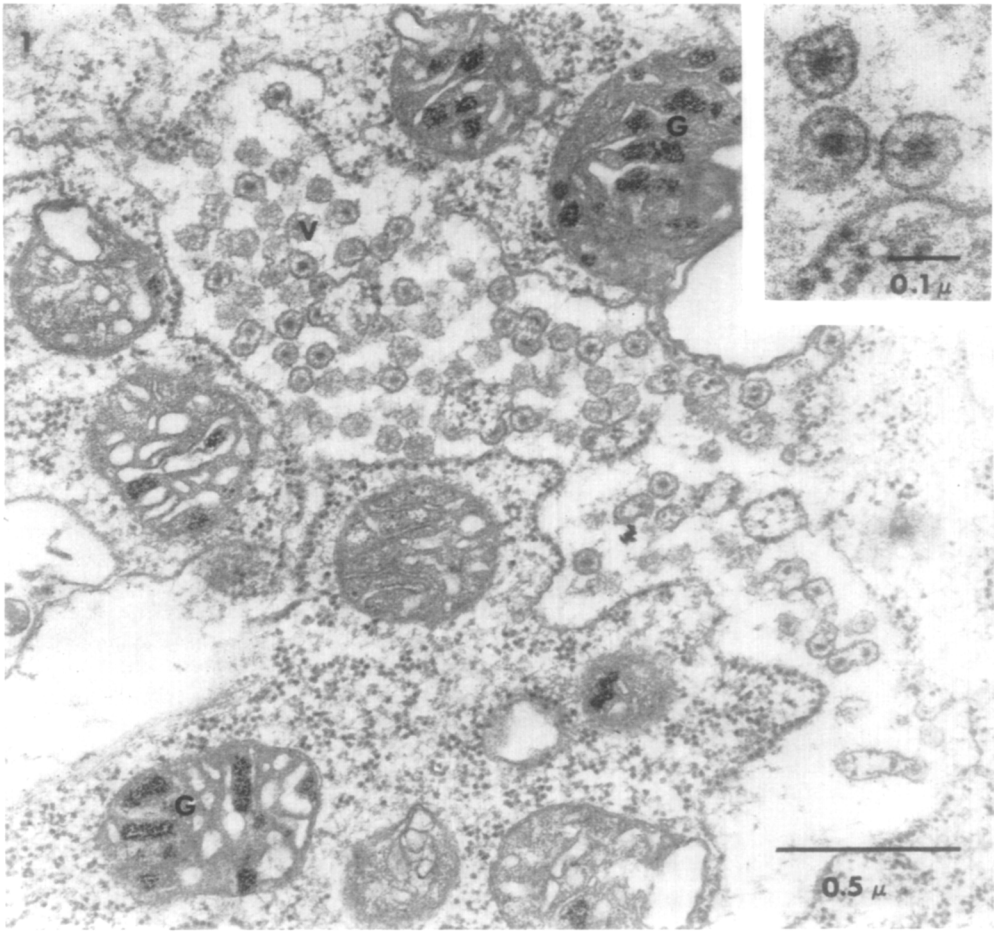


FIG. 1. Numerous type R virus-like particles (V) in the cisterna of the endoplasmic reticulum of an osmotically stressed BHK-21/4 cell. Intramitochondrial granules (G) are present. $\times 48,000$. Insert illustrates the radial structures of three particles. $\times 90,000$.

FIG. 2. Several type R particles in the cisterna of the endoplasmic reticulum of a BHK-21/4 cell. $\times 48,000$.

FIG. 3. A single type R particle associated with the nuclear envelope of a BHK-21/4 cell. The particles are characteristically associated with the endoplasmic reticulum and less frequently with the nuclear envelope. $\times 48,000$.

amination in a Philips EM 200 electron microscope.

As can be seen in Table 1, all clones of BHK-21 cells examined contain type R virus-like particles. These characteristic particles were found either in dilated cisternae of granular endoplasmic reticulum (Figs. 1 and 2) or associated with the nuclear envelope (Fig. 3). The particles have a diameter of 85–110 m μ , a nucleoid of 40–50 m μ , and a characteristic series of radial structures which taper as their distance from nucleoid increases (Fig. 1, insert). In the BHK-21/4 line, the number of particles per cell increased with passage of the cells. This phenomenon was also observed by Wheatley and Macpherson [unpublished observations quoted by Jarrett and Macpherson (14)] for BHK-21/13 cells. An apparent increase in the number of particles per cell could also be provoked by elevating the osmotic pressure of the medium with sucrose. The hypertonicity also caused an increase in intramitochondrial granules (Fig. 1). It has been suggested that in other cells (15) granules similar to those depicted are concerned with the regulation of the internal ionic environment of the mitochondrion. In contrast to the BHK-21 cells, no virus-like particles were seen either in HaK or RPMI-1846 cells.

Embryonic hamster kidney cells have been examined both directly and after subsequent culture *in vitro*. No virus-like particles were found. Similarly, a limited study of the germinal centers from Peyer's patches of adult hamsters failed to indicate the presence of any virus-like particles. Type R particles were readily found, however, in a SV-40-induced fibrosarcoma of a hamster.

Although type C and type A virus-like particles have been described in murine cell lines (16–18), type R particles have been found only in neoplastic hamster cells and are not seen in normal hamster cells. Because of the occurrence of type R particles in transformed hamster cells it is tempting to speculate as to the role these particles might play in the induction or expression of malignancy. However, without demonstrable biological activity it is impossible to assess the significance of these particles.

While this manuscript was in preparation, similar virus-like particles were detected in

an established bovine cell line (19). The particles were spherical with a diameter of 90 m μ and contained an electron-dense central core about 45 m μ in diameter, from which lines radiate to the outer border of the particle. The particles were frequently seen within the nuclear envelope or in cisternae of the endoplasmic reticulum. The characteristics of the bovine virus-like particle are essentially the same as those of the hamster particle and it would now appear that type R particles have been demonstrated in at least two distinct species.

ACKNOWLEDGMENTS

The senior author thanks Dr. William H. Murphy for the use of his laboratory facilities and his interest during the course of this study. The technical assistance of Mrs. Dorothy K. Reuss and Barbara T. Lamb is gratefully acknowledged. This investigation was supported in part by USPHS Grant 1-P13-DE-02731.

REFERENCES

1. MACPHERSON, I. and STOKER, M. *Virology* **16**, 147–151 (1962).
2. BERNHARD, W. and TOURNIER, P. *Ann. Inst. Pasteur* **107**, 447–455 (1964).
3. MCGEE-RUSSELL, S. M., VIZOSO, A. D. and SANDERS, F. K. *3rd Europ. Conf. Electron Microscop.*, Prague, pp. 367–368 (1964).
4. COMPANS, R. W., HOLMES, K. V., DALES, S. and CHOPPIN, P. W. *Virology* **30**, 411–426 (1964).
5. MURPHY, F. A., HALONEN, P. E. and HARRISON, A. K. *J. Virol.* **2**, 1223–1227 (1968).
6. THOMAS, J. A., DELAIN, É. and HOLLANDE, É. *Compt. Rend.* **264**, 785–788 (1967).
7. STENBACK, W. A., VAN HOOSIER, G. L., JR. and TRENTIN, J. J. *Proc. Soc. Exptl. Biol. Med.* **122**, 1219–1223 (1966).
8. STENBACK, W. A., VAN HOOSIER, G. L., JR. and TRENTIN, J. J. *J. Virol.* **2**, 1115–1121 (1968).
9. STOKER, M. and MACPHERSON, I. *Nature* **203**, 1355–1357 (1964).
10. HOLMES, K. V. and CHOPPIN, P. W. *J. Exptl. Med.* **124**, 501–520 (1966).
11. HALONEN, P., STEWART, J. and HALL, A. *Ann. Med. Exptl. Biol. Fenniae (Helsinki)* **45**, 182–185 (1967).
12. SHIPMAN, C., JR. *Proc. Soc. Exptl. Biol. Med.* **130**, 305–310 (1969).
13. MOORE, G. E., LEHNER, D. F., KIKUCHI, Y. and LESS, L. A. *Science* **137**, 986–987 (1962).

14. JARRETT, O. and MACPHERSON, I. *Intern. J. Cancer* 3, 654-662 (1968).
15. FAWCETT, D. W. *An Atlas of Fine Structure*. Saunders, Philadelphia, Pennsylvania (1966).
16. DALES, S. and HOWATSON, A. F. *Cancer Res.* 21, 193-197 (1961).
17. KINDIG, D. A. and KIRSTEN, W. H. *Science* 155, 1543-1545 (1967).
18. CROMACK, A. S. *J. Gen. Virol.* 2, 195-198 (1968).
19. MUSSGAY, M., RECZKO, E. and AHL, R. *J. Gen. Virol.* 4, 445-447 (1969).

CHARLES SHIPMAN, JR.
GRETCHEN C. VANDER WEIDE
BOOE IL MA¹

*Department of Oral Biology
School of Dentistry and
Department of Microbiology
School of Medicine
The University of Michigan
Ann Arbor, Michigan 48104*

Accepted May 20, 1969

¹Public Health Special Fellow (No. FO3 DE42829-01).

Selective Failure of Protein Synthesis in Herpesvirus-Infected Cells Deprived of Arginine¹

The yield of several DNA viruses (1-4), including that of herpes simplex virus (5-8), from animal cells in continuous cultivation is largely dependent on the presence of arginine in the medium. The requirement for arginine is more stringent than that for other amino acids (5) but it is not uniform; the yield of herpes simplex virus from primary cultures of human embryonic fibroblasts, chick embryo fibroblasts, or of monkey kidney cells is unaffected by arginine deprivation (6).

The differences between primary and continuous cell cultures with respect to the capacity to support virus multiplication in arginine-free medium may reflect the size of the arginine pool (9-11). Arginine starvation of cells in continuous cultivation does not

prevent adsorption, penetration, uncoating of herpes simplex virus, and reproductive events occurring during the first several hours after infection. The latter include the synthesis of viral DNA (9) and the synthesis of viral proteins and their transport from cytoplasm into the nucleus as evidenced by the presence of viral antigens in both compartments of the cell (8). However, immunofluorescent granules in the cytoplasm and nucleus and viral particles are not made (8). These findings suggest that arginine deprivation causes a selective decrease in the synthesis of some viral macromolecules.

One hypothesis that could explain these findings is based on the assumption that in infected cells depleted of arginine the translation of mRNA comes to a halt at codons specifying arginine. It could be expected therefore that in arginine-depleted cells the probability that a given peptide is completed would be inversely proportional to its molecular weight and to its arginine content. The hypothesis is of particular interest in view of the evidence presented by us and others that herpesvirus contains proteins ranging up to 125,000 daltons (12, 13). To test this hypothesis we compared the size distribution of proteins made in untreated and arginine-depleted infected cells. In these experiments replicate HEp-2 cell cultures, each containing 2×10^7 cells, were exposed for 1 hour at 37° to sufficient virus to yield a multiplicity of 50 plaque-forming units per cell. One set of cultures (control) was then overlaid with mixture 199 containing 1% dialyzed calf serum (199-1). The other set was overlaid with the same medium but lacking arginine (199-1 arg⁻). At 6 hours post-infection the maintenance medium was decanted, the cells were washed, and replenished as follows: Control cultures received medium 199-1 lacking lysine (199-1 lys⁻) but supplemented with 0.2 μCi of ¹⁴C-1-lysine per ml of medium. The arginine-deprived cultures were replenished with medium 199-1 arg⁻lys⁻ supplemented with 2 μCi of ³H-1-lysine per ml of medium. The final concentration of lysine in the two sets was approximately the same. The cells were then reincubated. The time of labeling was based on the observations that host polyribosomes

¹Aided by grants from the National Cancer Institute, U. S. Public Health Service (CA 08494), the American Cancer Society (E 314E), and the National Science Foundation (GB 8242).